SOP-P066

Hypercyte/HyperView Analysis

Well Analysis, Fluorescence Pre-Analysis
for Redox (Calcein-MBBR-Mitosox) and JC-1

NOTE: On the HyperCyte System, use the following naming scheme:

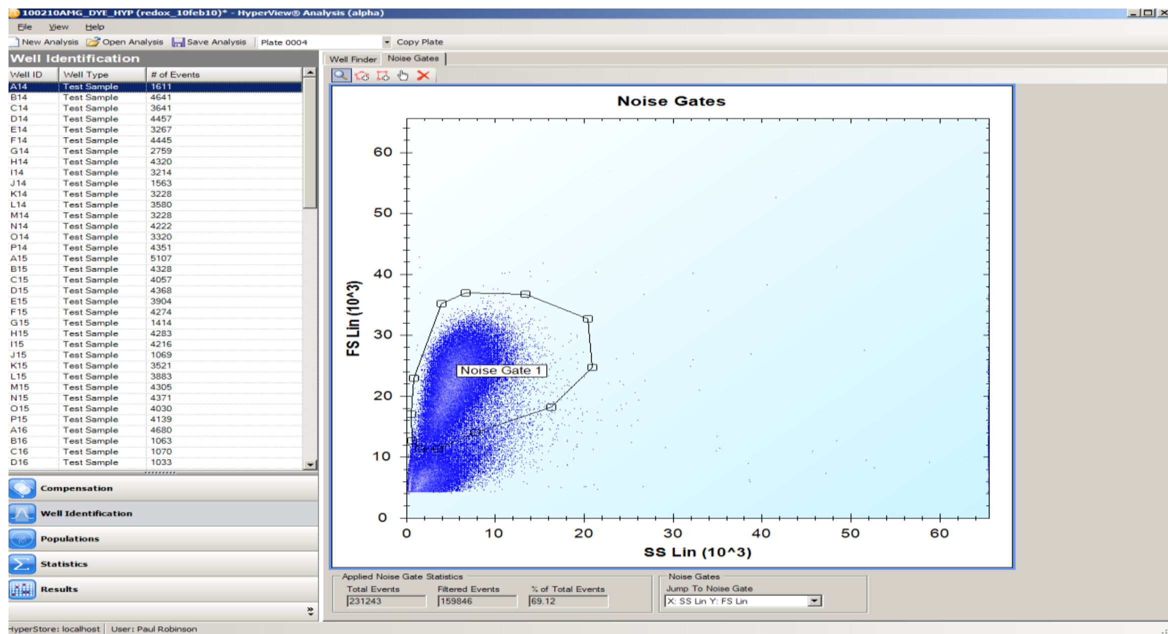
1. HyperView Designer EXPERIMENT Name Format: **20121024r (or j)** (YYYMMDD)
2. PLATE NAME FORMAT: **p0001a**
3. HyperView ANALYSIS Name Format: **AM2r (or j)**

Well Analysis:

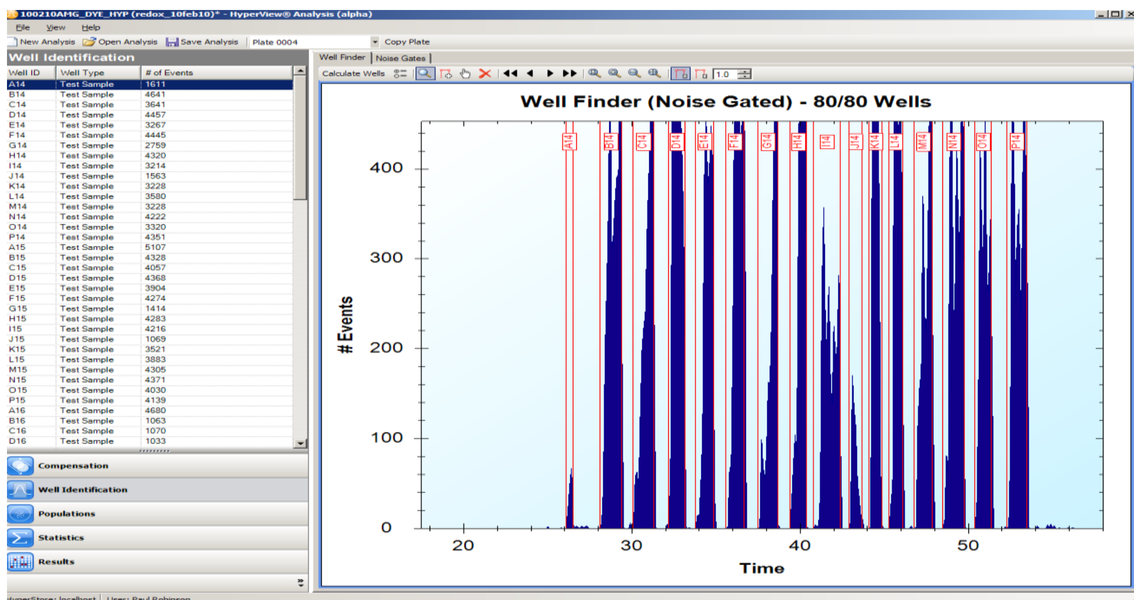
1. Open **HyperView Analysis**
2. Click on **New Analysis** tab at the top
3. Browse for your experiment, open it
4. Give the analysis the name: **AM2r (or j)**
5. Click on **Create**
6. Select the plate you wish to analyze
7. Click on the **NOISE GATE** button (this only assists the well identification)
8. Set a noise gate around the “good” cells
9. Click on the **WELL FINDER** button
10. Click on the **CALCULATE WELLS** button

Creating the Noise Gate

- The noise gate is selecting only data that should be considered in the TIME gates. Any data outside the noise gate will NOT be included in selection of TIME gates



- Carefully check the TIME gates. There should be the exact number of time gates as there are total wells in the assay. Review the entire plate. This must be done for EVERY plate. Gates may need to be edited, created, or deleted.



Exporting “All Well FCS data”

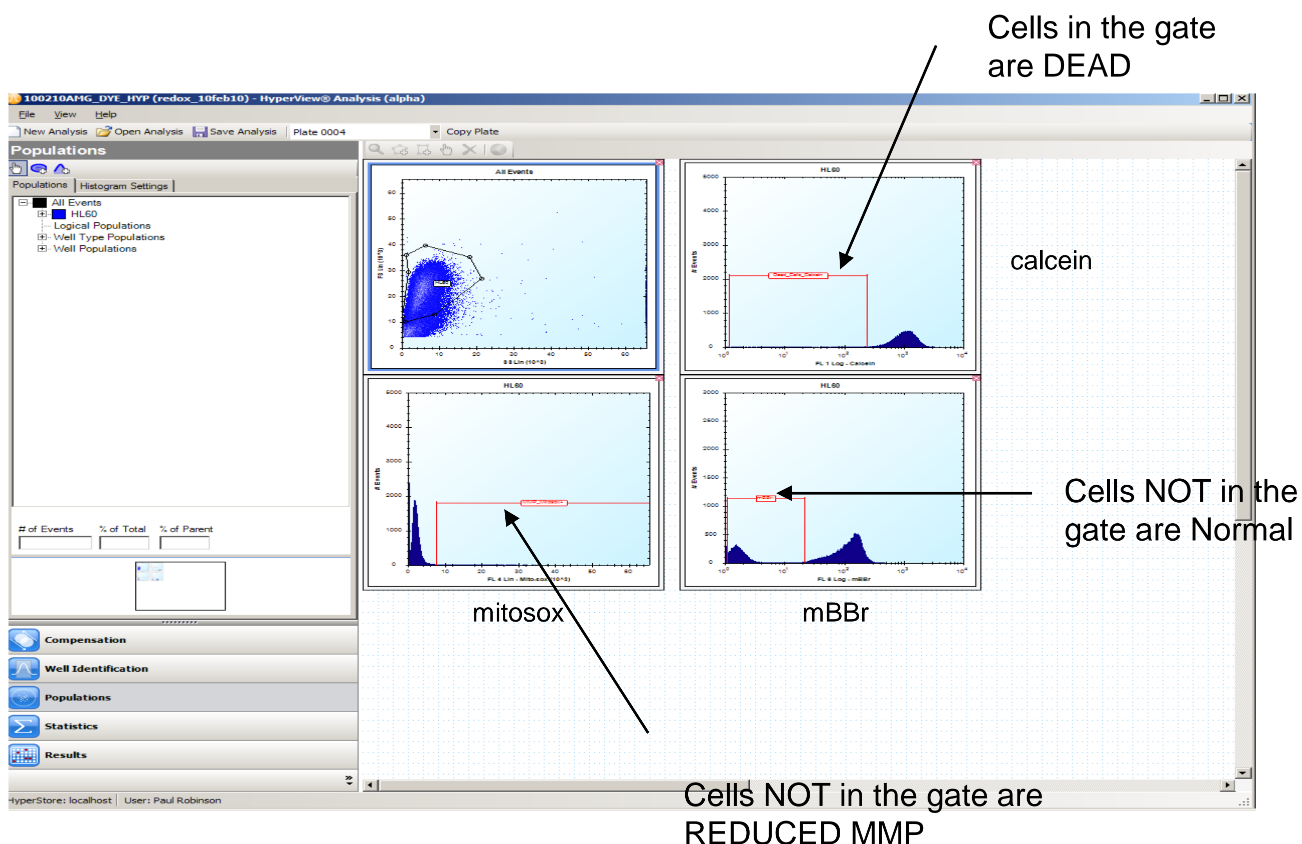
- Go to: file>export> export all well FCS data
- Select the file path to save this in:
  - Scratch/.projects/AMG2 or Amgen HL60 screens or whatever ti may be
  - Create a folder with the date in the format 20121024 (YYYMMDD)
  - Open this folder
  - Type the appropriate filename i.e. 20121024j-AM2j-p0001a
  - (or copy and paste)
  - Select SAVE

Redox Analysis
(Calcein-Mitosox-mBBr)

1. Create a light scatter dot plot for gating purposes:
   -click the **Populations** mode button on bottom left
   -click on the dot plot icon, then into the workspace (a two parameter dot plot will appear)
2. Change the x-axis to SS by clicking on the current label of that axis, a drop down menu will appear of the choices available

3. Create a light scatter gate and rename it **HL60**
4. Create single parameter histograms for each of the three fluorochromes
   -click on the histogram icon, then click three times in the workspace (three histograms should appear)
5. Gate the histograms on the HL60 light scatter gate
6. Change the x-axis labels as above to make one histogram for each fluorochrome. – calcein, mitosox, and mBBr
7. Create analysis gates for each histogram as shown in the diagram on the next page.
8. Label these gates as follows:
   1. For calcein label as: **Dead Cells_Calcein**
   2. For mitosox label as: **MMP_Mitosox+**
   3. For mBBr label as: **mBBr-**

1. **Dead Cells_Calcein** cells in the gate = dead cells
2. **MMP_MITOSOX+** cells in the gate = increase in MMP
3. **MBBr(-)** cells in the gate= loss of MMP



Create the Custom Parameters for Exportation

- Click on the **Statistics** mode button
- Under the Standard tab, check the HL60 gate box, then check the box for event count/count
- Click on **Apply**
- select the **Custom** tab, then click on the **Add** button
- on **Add Population Ratio** window click on the down arrow for population
-select the first one which should be calcein
-for the **"as % of population"** select HL60
-repeat this to creat similar parameters for both mitosox and MBBr
-save this with the exact name as the plate, but add _STAT at the end of the filename (20121024j-AM2j_p0001a_STAT)

Click CUSTOM and make the 3 datasets

These are the columns to export to XL

100210AMG_DYE_HYP (redox_10feb10) - HyperView® Analysis (alpha)

File View Help

New Analysis | Open Analysis | Save Analysis | Plate 0004 | Copy Plate

Statistics

Standard | Custom | Settings

Population Ratios

Dead_Cells_Calcein as % of HL60
mBBr- as % of HL60
MMP_Mitosox+ as % of HL60

Add... | Edit... | Delete

Compensation
Well Identification
Populations
Statistics
Results

Population Statistics | Well Statistics

Excel Export

Well ID	Well Type	Dead_Cells_Calcein as % of HL60	mBBr- as % of HL60	MMP_Mitosox+ as % of HL60
A14	Test Sample	87.44	67.63	66.67
B14	Test Sample	3.13	.33	3.18
C14	Test Sample	2.53	0	2.69
D14	Test Sample	6.50	.16	2.72
E14	Test Sample	2.08	.04	2.48
F14	Test Sample	2.92	.13	2.97
G14	Test Sample	2.33	0	2.90
H14	Test Sample	3.46	0	1.88
I14	Test Sample	2.37	.26	2.75
J14	Test Sample	98.61	1.08	35.96
K14	Test Sample	3.02	.07	2.36
L14	Test Sample	5.57	.04	3.53
M14	Test Sample	2.39	.07	2.86
N14	Test Sample	3.46	.03	3.63
O14	Test Sample	2.49	.07	2.63
P14	Test Sample	2.87	0	3.06
A15	Test Sample	2.99	4.33	2.80
B15	Test Sample	2.28	.47	2.33
C15	Test Sample	3.34	.18	2.86
D15	Test Sample	3.26	.28	3.42
E15	Test Sample	2.84	.58	3.23
F15	Test Sample	2.28	.11	2.59
G15	Test Sample	93.77	.37	68.13
H15	Test Sample	2.58	.03	2.72
I15	Test Sample	4.79	.09	5.47
J15	Test Sample	97.67	.47	33.49
K15	Test Sample	6.09	.18	4.69
L15	Test Sample	2.57	.03	2.08
M15	Test Sample	2.04	.08	2.67
N15	Test Sample	2.84	.05	2.79
O15	Test Sample	2.58	0	2.56
P15	Test Sample	3.14	.03	2.79
A16	Test Sample	4.71	6.83	4.66
B16	Test Sample	91.95	6.90	25.29
C16	Test Sample	100.00	4.00	24.00
D16	Test Sample	100.00	3.23	9.68
E16	Test Sample	96.74	2.17	17.39
F16	Test Sample	98.59	12.68	8.45
G16	Test Sample	95.95	0	10.81
H16	Test Sample	97.44	5.13	5.13
I16	Test Sample	4.43	.11	2.39
J16	Test Sample	100.00	4.08	14.29
K16	Test Sample	100.00	0	16.22
L16	Test Sample	100.00	0	20.00
M16	Test Sample	97.26	12.33	12.33
N16	Test Sample	92.31	5.77	3.85
O16	Test Sample	6.59	0	2.21
P16	Test Sample	3.05	0	2.27

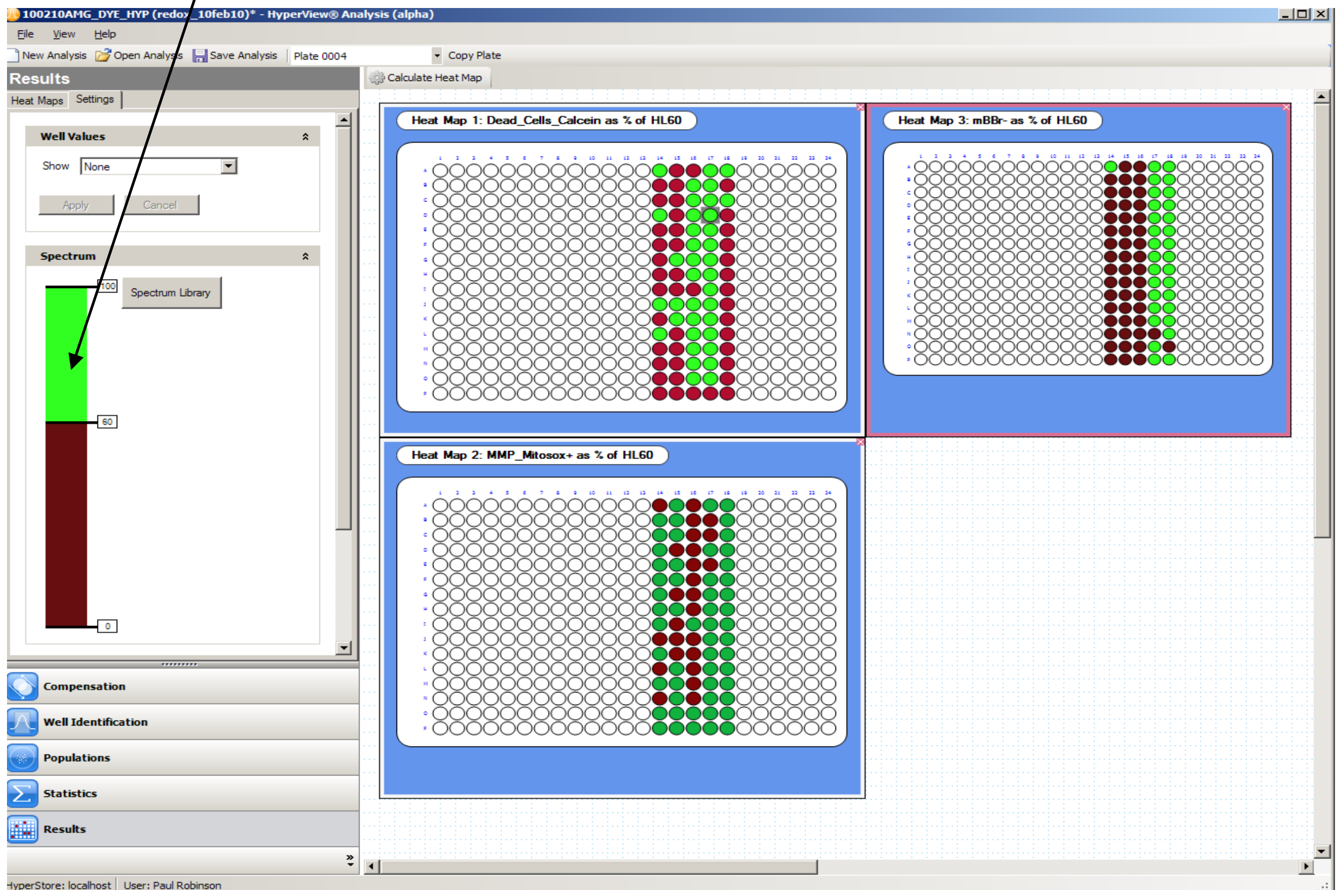
HyperStore: localhost | User: Paul Robinson

Create the HEAT MAPS

– There are 3 separate maps – one for each fluorochrome of the assay MITOSOX, MBBR and CALCEIN. This is simply a visualization of the data.

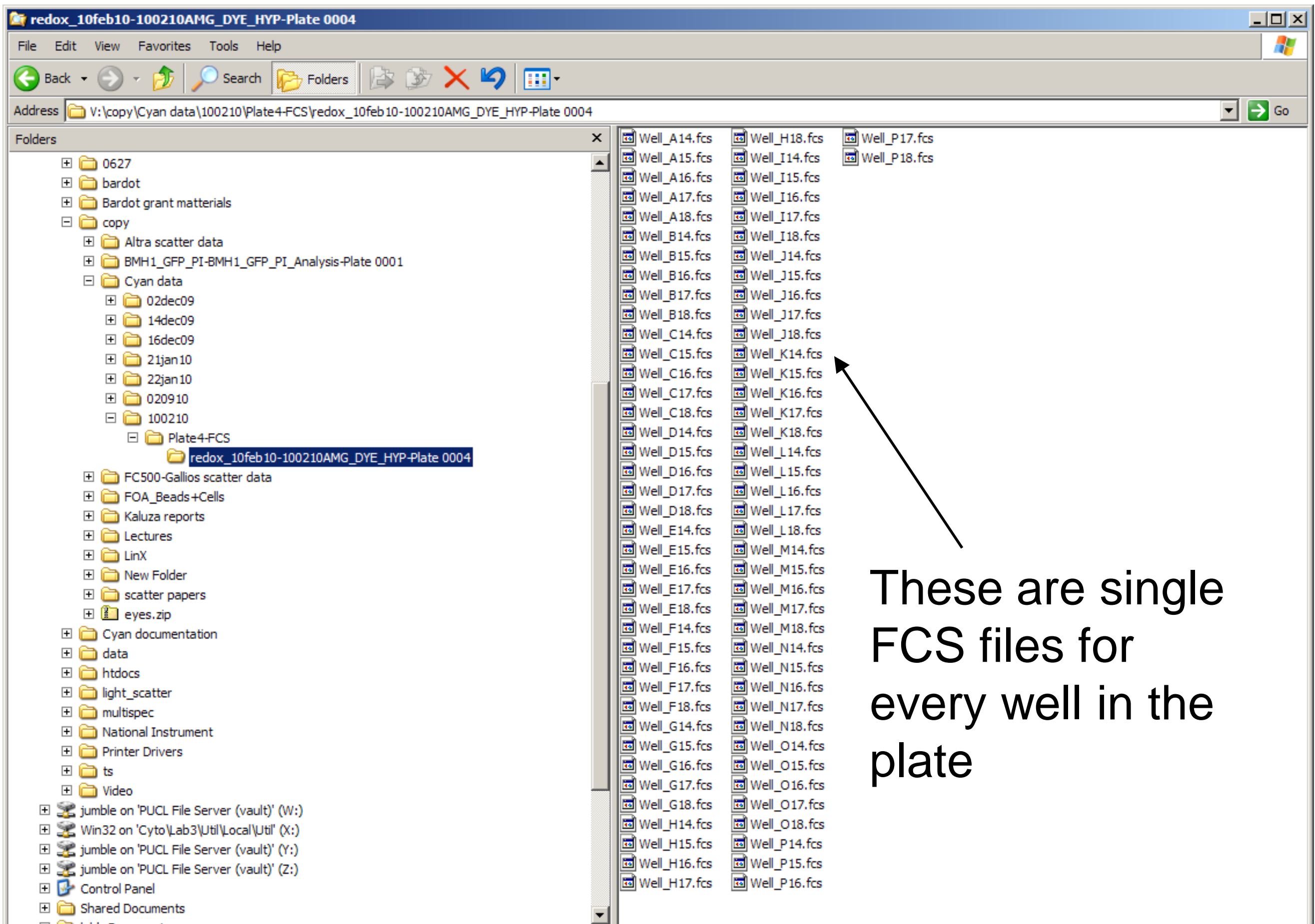
Select the **Results** mode button
Click on the **Add** tab
Add Heat Map box appears
Under **STATISTIC** select parameter for heat map, select calcein first
Make two more, one each for mitosox and mBBr
Select **Settings** tab
Select a heat map
Under **well values** select "**show normalized values**", click **apply**
Select **Spectrum Library**, select **2 color**, and **apply** spectrum
Go to **User Define Range**, check **enable**, set **min=0** and **max=100, click apply**
Determine where to move the spectrum slider
SAVE THE ANALYSIS

Set the colors based on +iv and –ives – These can be set separately for each graph



Archive and document the data storage

- Use the naming conventions we established.



Repeat the analysis procedure for all plates if the same experiment. See the process below for copying a plate analysis format from one plate to another.

1. Once the analysis process is complete, you must go back and check
2. The noise gate for each plate
3. The time gates for all wells.
4. Ensure that the gated populations and the analysis populations are still correct.
5. Resave the analysis
6. Create the XL files, and then create the FCS files for each well.

Select COPY PLATE

100210AMG_DYE_HYP (redox_10feb10) - HyperView® Analysis (alpha)

File View Help

New Analysis | Open Analysis | Save Analysis | Plate 0004 | Copy Plate

Statistics

Standard | Custom | Settings

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Add... | Edit... | Delete

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Copy Plate

Copy Plate 0004 to:

- Plate 0001
- Plate 0002
- Plate 0003
- Plate 0005

OK | Cancel

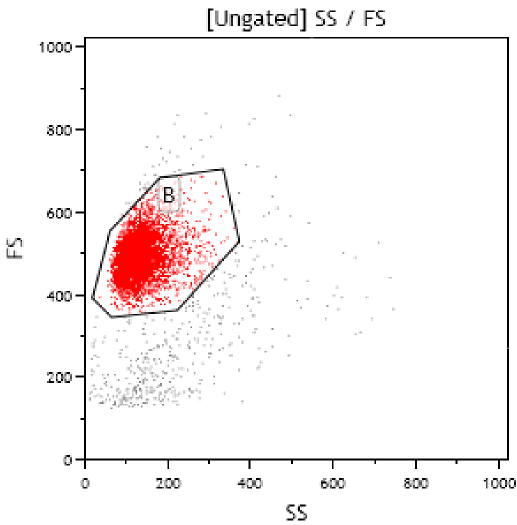
Enter the plate to copy to here

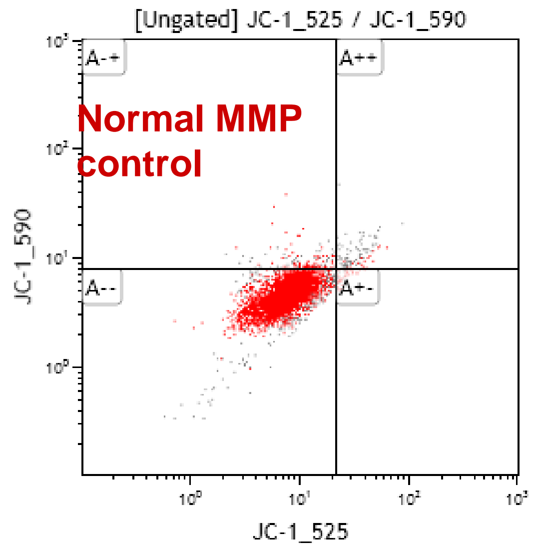
HyperStore: localhost | User: Paul Robinson

Start | Live365 - Player Window... | 100210AMG_DYE_HYP (r... | Microsoft PowerPoint ... | 100210 | 10:58 AM

JC-1 Analysis

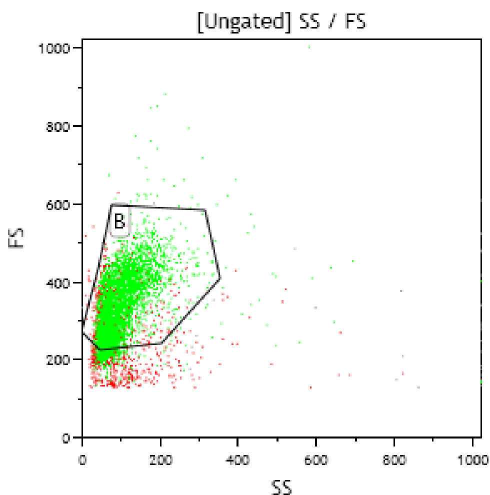
1. Establish the live gate of cells first (HL60)
2. WE can create either a QUAD stat or 2 gates – I prefer 2 gates
3. The dataset will plot the % of cells that move from Gate C to gate D
4. This is MMP+ cells to MMP- cells



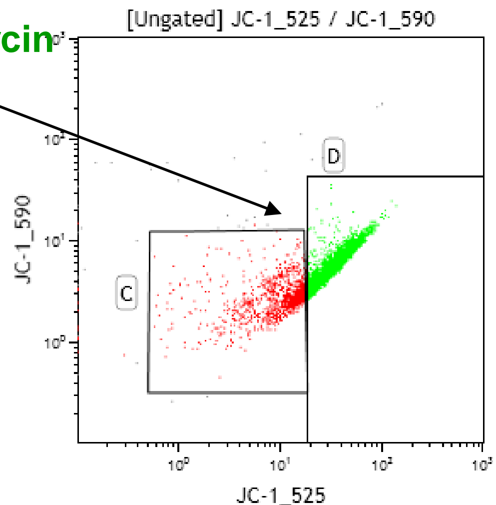


Gate	Number	%Gated
All	5,711	100.00
A--	5,413	94.78
A-+	153	2.68
A+-	49	0.86
A++	96	1.68

Valinomycin control



Gate	Number	%Gated
All	6,225	100.00
B	5,231	84.03



Gate	Number	%Gated
All	6,225	100.00
C	1,233	19.81
D	4,815	77.35