SOP-P066

<u>Hypercyte/HyperView Analysis</u> Well Analysis, Fluorescence Pre-Analysis for Redox (Calcein-MBBR-Mitosox) and JC-1

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

- 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

<u>Redox Analysis</u> (Calcein-Mitosox-mBBr)

- 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
- 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 X								
0210AMG_DYE_HYP (/edox_10feb10) - HyperView® Ana	alysis (alpha)					_			
View Help					\backslash				
Analysis 💕 Open Analysis 🔚 Save Analysis 🛛 Plate 0004	-	Copy Plate			\mathbf{i}				
tistics	Description Onti	Wall Statistics			\sim				
istics	Population Stati	sucs [Weir Statistics]		- 1	\				
ard Custom Settings	Excel Export	t		1					
nulation Ratios	Av/all ID	Vell Tures	Deed Calls Calasia as % of bil 60	PPPs as % of ULCO	MMD Milesen as % of ULCO				
	Well ID	Well Type	Dead_Cells_Calcell as % of HL60	mbbr- as % of HLbU	MMP_Mitosox+ as % of HL60				
ad_Cells_Calcein as % of HL60	AI4	Test Sample	87.44	67.63	bb.b/				
hebr-as , or HLSU MMP_Mitosox+ as % of HLGO	D14	Test Sample	3.13	.33	3.10				
	D14	Test Sample	2.00	16	2.05				
	D14	Test Sample	2.00	. 10	2.72				
	C14	Test Sample	2.00	10	2.40				
	G14	Test Sample	2.02	0	2.07				
	H14	Test Sample	3.46	0	1.90				
	114	Teet Sample	2 37	26	2.75				
	114	Test Sample	98.61	1.08	35.96				
	K14	Test Sample	3.02	07	2 36				
	1 14	Test Sample	5.02	.04	2.50				
	M14	Test Sample	2 39	07	2.86				
	N14	Test Sample	2.55	.07	2.00				
	014	Test Sample	2.49	07	2.63				
	D14	Test Sample	2.45	.07	2.05				
	A15	Test Sample	2.07	4 22	2 90				
	P15	Test Sample	2.55	4.55	2.00				
	C15	Test Sample	2.20	18	2.55				
	D15	Test Sample	3.24	28	3.42				
	F15	Test Sample	2.84	58	3.72				
	E15	Test Sample	2.04	11	2.59				
	G15	Test Sample	93.77	37	68.13				
	H15	Test Sample	2.58	03	2 72				
	115	Test Sample	4 79	09	5 47				
	.115	Test Sample	97.67	47	33.49				
	K15	Test Sample	6.09	18	4.69				
	115	Test Sample	2.57	03	2.08				
	M15	Test Sample	2.04	.08	2.67				
	N15	Test Sample	2.84	.05	2.79				
	015	Test Sample	2.58	0	2.56				
Add Edit Delete	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				
Companyation	D16	Test Sample	100.00	3.23	9.68				
compensation	E16	Test Sample	96.74	2.17	17.39				
	F16	Test Sample	98.59	12.68	8.45				
Well Identification	G16	Test Sample	95.95	0	10.81				
	H16	Test Sample	97.44	5.13	5.13				
Populations	116	Test Sample	4.43	.11	2.39				
	J16	Test Sample	100.00	4.08	14.29				
Chabieries	K16	Test Sample	100.00	0	16.22				
Statistics	L16	Test Sample	100.00	0	20.00				
	M16	Test Sample	97.26	12.33	12.33				
Results	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				

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**

Include: Unit Hole

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.

😂 redox_10feb10-100210AMG_DYE_HYP-Plate 0004				
File Edit View Favorites Tools Help				Ar
🚱 Back 🔹 🕥 - 🏂 🔎 Search 💦 Folders 🕼 🕉 🗙 🌳 📰 -				
Address 🛅 V:\copy\Cyan data\100210\Plate4-FCS\redox_10feb10-100210AMG_DYE_HYP-Plate 0004				💌 🄁 Go
Folders	×	Well_A14.fcs	Well_H18.fcs	Well_P17.fcs
E 🚞 0627	•	Well_A15.fcs	Well_I14.fcs	Well_P18.fcs
🕀 🧰 bardot		Well_A 16.fcs	well_115.fcs	
🕀 🛅 Bardot grant matterials		Well_A17.fcs	Well_116.fcs	
		Well_A18.rcs	Well_117.fcs	
🗄 🧰 Altra scatter data		Well B15 fcs	Well_114.fcs	
Emeril GFP_PI-BMH1_GFP_PI_Analysis-Plate 0001		Well B16.fcs	Well 115 fcs	
🖃 🦲 Cyan data		Well B17.fcs	Well J16.fcs	
E C 14dec00		Well B18.fcs	Well J17.fcs	
E C 16dec09		Well_C14.fcs	Wel J18.fcs	
1 (100c0) 1 (100c0)		Well_C15.fcs	Well_K14.fcs	
1 22jan10		🖻 Well_C 16.fcs	🚾 Well_K15.fcs	₹
E		🖬 Well_C17.fcs	🖬 Well_K16.fcs	$\mathbf{\lambda}$
□ <u>□</u> 100210		Well_C18.fcs	🖬 Well_K17.fcs	$\mathbf{\lambda}$
Plate4-FCS		Well_D14.fcs	🔤 Well_K18.fcs	$\mathbf{\lambda}$
redox_10feb10-100210AMG_DYE_HYP-Plate 0004		Well_D15.fcs	🛅 Well_L 14.fcs	$\mathbf{\lambda}$
🗉 🚞 FC500-Gallios scatter data		Well_D16.fcs	Well_L15.fcs	$\mathbf{\lambda}$
🗉 🧰 FOA_Beads+Cells		Well_D17.fcs	Well_L16.fcs	$\mathbf{\lambda}$
🗉 🗀 Kaluza reports		Wel_D18.fcs	Wel_L17.fcs	$\mathbf{\lambda}$
🗉 🗀 Lectures		Well_E14.fcs	Well_L18.fcs	$\mathbf{\lambda}$
🕀 🚞 LinX		Well_E15.fcs	Well_M14.fcs	
🗉 🧰 New Folder		Well_E16.fcs	Well_M15.fcs	Those are single
scatter papers		Well_E17.fcs	Well_M10.rcs	These are single
🗄 🟥 eyes.zip		Well_E14 fcc	Well_M19 fcs	
🗄 🧰 Cyan documentation		Well F15 frs	Well N14 frs	FCS files for
🗈 🛄 Gata		Well F16.fcs	Well N15.fcs	
E intoocs		Well F17.fcs	Well N16.fcs	
		Well_F18.fcs	Well_N17.fcs	every well in the
		Well_G14.fcs	wel_N18.fcs	
T Printer Drivers		🖻 Well_G15.fcs	🖬 Well_014.fcs	nlata
T 🗎 ts		🖬 Well_G16.fcs	🖬 Well_015.fcs	plate
🗉 🧰 Video		Well_G17.fcs	🔟 Well_016.fcs	· · · · · ·
		Well_G18.fcs	Well_017.fcs	
⊞ See Win32 on 'Cyto\Lab3\Util\Local\Util' (X:)		Well_H14.fcs	Well_018.fcs	
🗉 🧝 jumble on 'PUCL File Server (vault)' (Y:)		Well_H15.fcs	Well_P14.fcs	
🗉 🛫 jumble on 'PUCL File Server (vault)' (Z:)		Well_H16.fcs	Well_P15.fcs	
🗉 📴 Control Panel		Well_H17.fcs	well_P16.fcs	
🗄 🛅 Shared Documents	-			

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.

Select COPY PLATE

- 5. Resave the analysis
- 6. Create the XL files, and then create the FCS files for each well.

210AMG_DYE_HYP (redox_10feb10) - HyperVi	iew® Analysis (alpha)	_/				_
View Help		\checkmark				
Analysis 🔂 Open Analysis 🔚 Save Analysis 🏼 Pla	ate 0004 • Co	py Plate				
stics	Population Statistic	well Statistics				
rd Custom Settings	Evcal Evcot	1				
		_		1		
pulation Ratios	Well ID	Well Type	Dead_Cells_Calcein as % of HL60	mBBr- as % of HL60	MMP_Mitosox+ as % of HL60	
ad Cells Calcein as % of HL60 Brase % of HL60	A14	Test Sample	87.44	67.63	66.67	
IP_Mitosox+ as % of HL60	614	Test Sample	3.13	.33	3.18	
-	D14	Test Sample	2.03	10	2.69	
	E14	Test Sample	2.00	.10	2.72	
	E14	Test Sample	2.00	13	2.40	
	G14	Test Sample	2.33	0	290 -	
	H14	Test Sample	346	0	1.88	
	114	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		.04	3.53	
	M14	Test Sample	Copy Plate	.07		r the plate
	N14	Test Sample	Copy Plate 0004 to:	83	3.63	i uio piato
	014	Test Sample	Plate 0001	.07	2.63	
	P14	Test Sample	Plate 0002	0	3.06 CODV	to horo
	A15	Test Sample	Plate 0003	4.33	2.80 UUUV	
	B15	Test Sample	Plate 0005	.47	2.33	
	C15	Test Sample	st Sample st Sample st Sample st Sample	.18	2.86	
	D15	Test Sample		.28	3.42	
	E15	Test Sample		.58	3.23	
	F15	Test Sample		.11	2.59	
	G15	Test Sample		.37	68.13	
	H15	Test Sample		.03	2.72	
	115	Test Sample		.09	5.47	
	J15	Test Sample		.47	33.49	
	K15	Test Sample		.18	4.69	
	L15	Test Sample	OK Cancel	.03	2.08	
	M15	Test Sample		.08	2.6/	
	N IS	rest Sample	2.04	cu.	2./3	
	015	Test Sample	2.00	02	2.00	
Add Edit Delete	A16	Test Sample	4 71	6.93	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	
Compensation	E16	Test Sample	96.74	2.17	17.39	
	F16	Test Sample	98.59	12.68	8.45	
Well Identification	G16	Test Sample	95.95	0	10.81	
	H16	Test Sample	97.44	5.13	5.13	
Populations	116	Test Sample	4.43	.11	2.39	
	J16	Test Sample	100.00	4.08	14.29	
Statistics	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	
esuits	N16	Test Sample	92.31	5.77	3.85	
	016	Test Sample	6.59	0	2.21	

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

