

APRIL 2002 -  
JUNE 2002

1. Dye transfer
2. DiI Studies
3. Bystander Effects  
(supernatant transfer)
4. Co-culture
  - DNA
  - DiI

GERASHCHENKO B.I

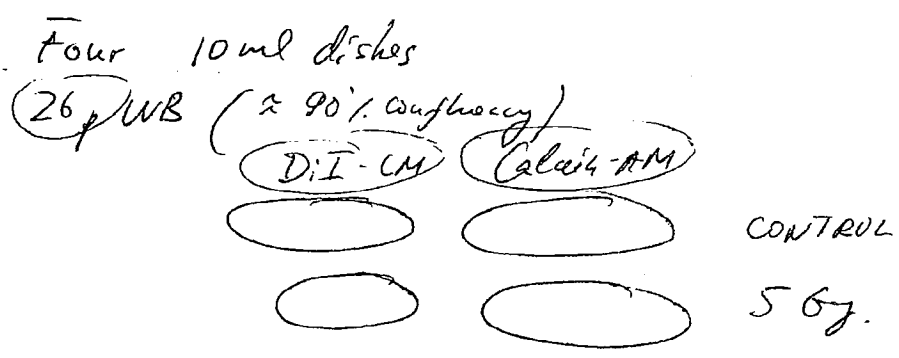
2x6 Transfer



50/50

April 2, 2002

CELL-TO-CELL COMMUNICATION +  
IRRADIATION (5 Gy) OF WHOLE POPULATION



IRRADIATION TIME: 11:20

STAINING PROTOCOL (see protocol of March 31, 2002)

10ml suspension of cells. ←

Resuspend in 5ml D-medium prior to plating

Counter Counts: 1083  
 1107 }  $\approx 1097 \times 400 = 4.4 \times 10^5$  cells/ml  
 1100 }

→ plate into 5ml dishes as follows:

(total # per dish  $\approx 3 \times 10^6$  cells) [Time: 12:00]

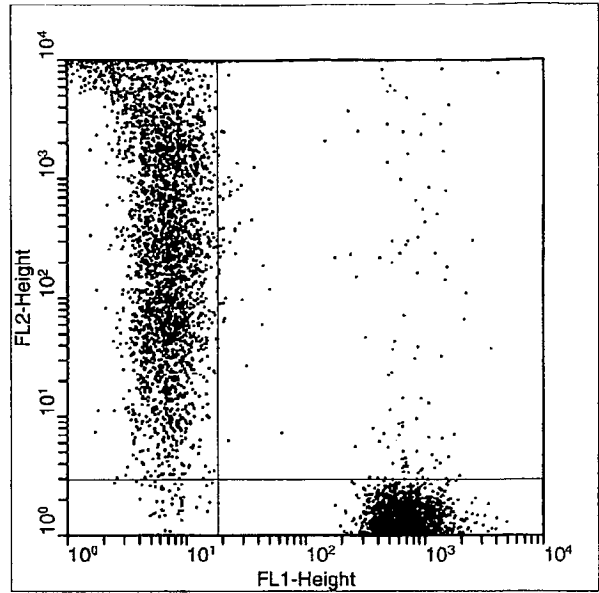
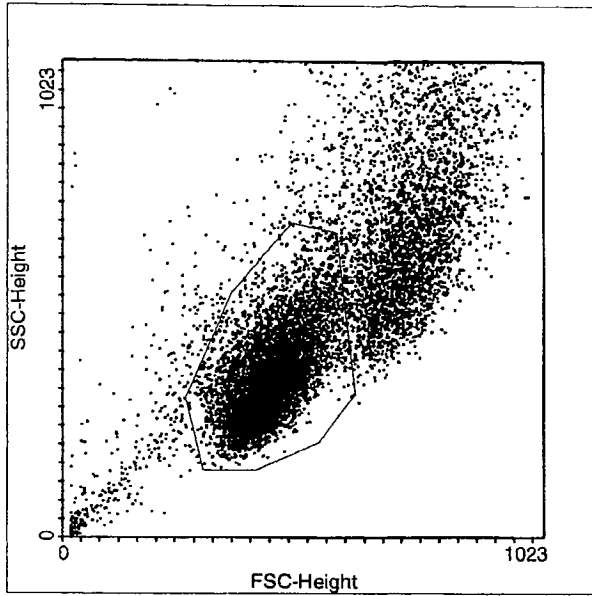
Add D-medium:  $\approx 2$  ml

1.7ml ( $\approx 1.5 \times 10^6$ ) cont. DiI stained, labeled cells +

1.7ml ( $\approx 1.5 \times 10^6$ ) cont. Calcein labeled cells

Co-culture time: 5h.

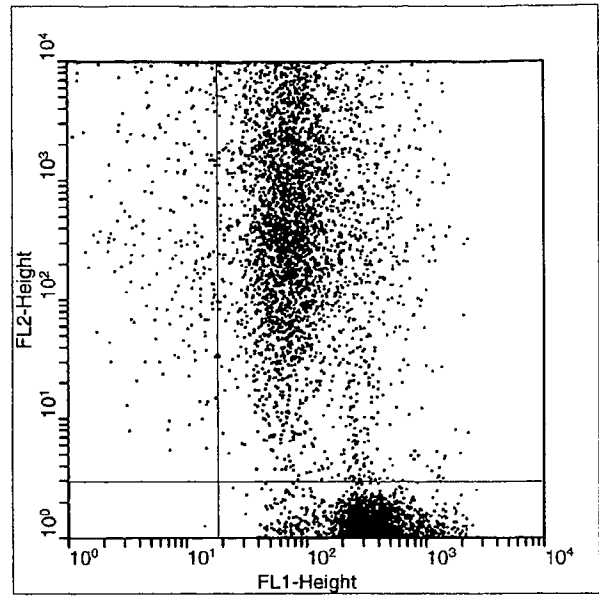
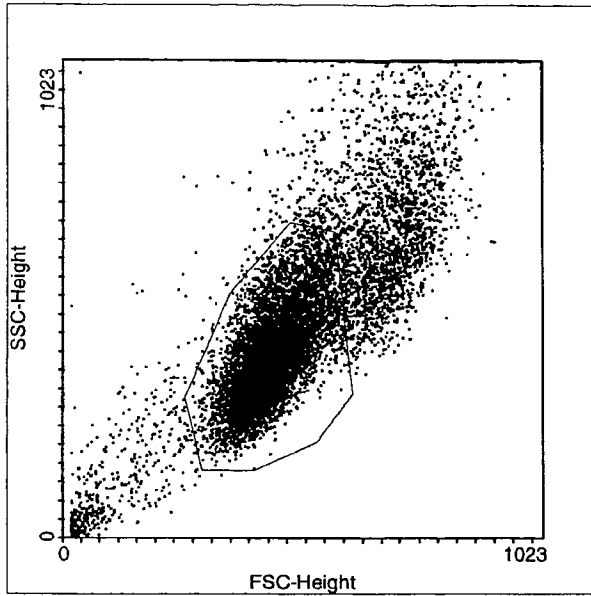
Next day (24h.?)



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 03 15:09:15 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040402 Sample: 0Gy Oh (control) 50/50  
 Date: 3-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 11680 58.40%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 324,120  

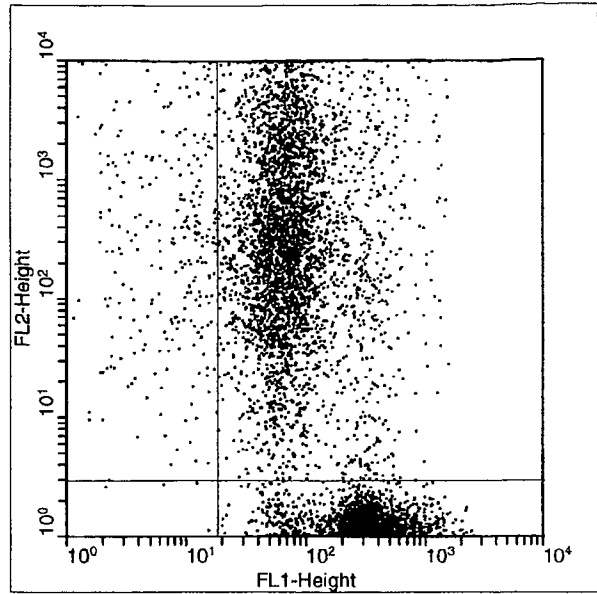
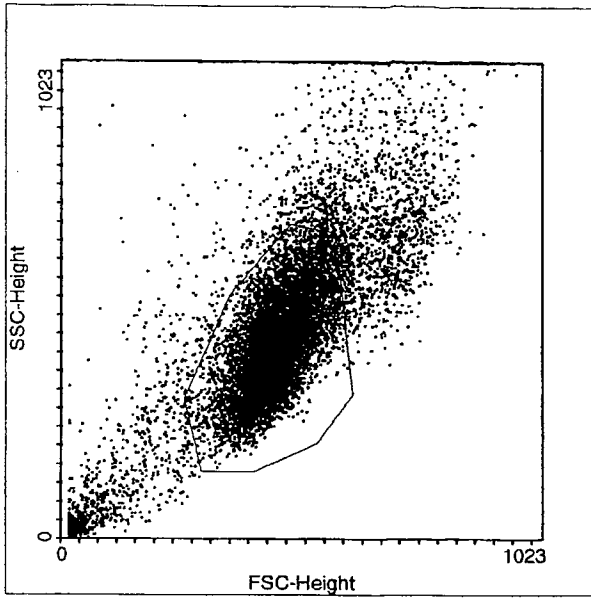
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	6.4	331.8	5812	29.06	49.76
2 UR	230.4	103.9	283	1.42	2.42
3 LL	7.0	1.9	86	0.43	0.74
4 LR	759.3	1.2	5499	27.50	47.08





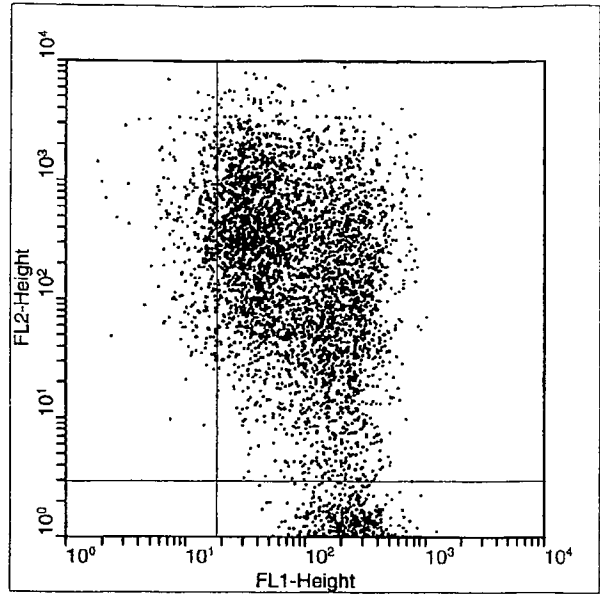
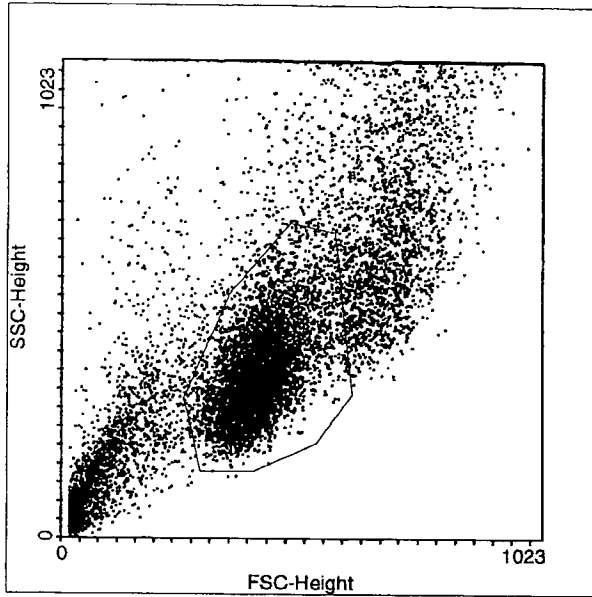
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 03 15:11:08 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040402 Sample: 0Gy 5h 50/50  
 Date: 3-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14439 72.19%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 324,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	6.7	577.6	455	2.28	3.15
2 UR	92.6	388.5	6785	33.93	46.99
3 LL	11.3	1.8	3	0.02	0.02
4 LR	360.9	1.2	7196	35.98	49.84



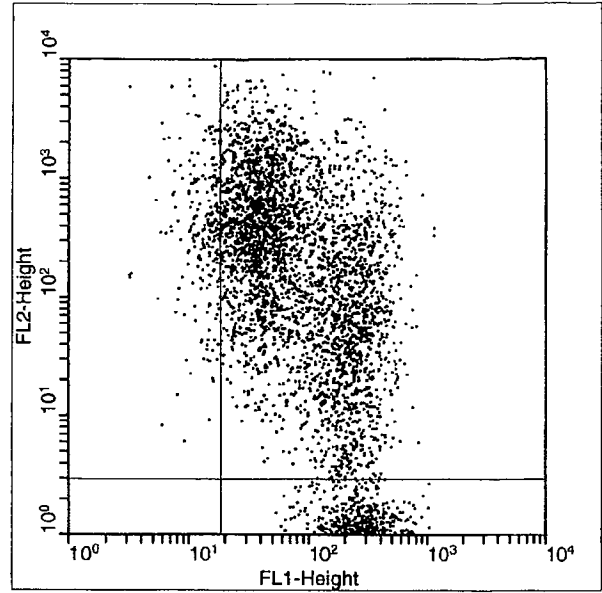
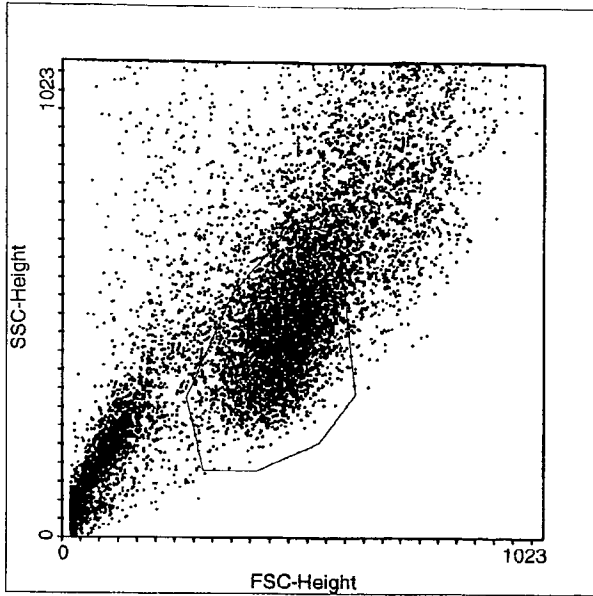
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 03 15:12:03 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040402 Sample: 5Gy 5h 50/50  
 Date: 3-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14861 74.31%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 324,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	6.5	357.9	534	2.67	3.59
2 UR	80.7	299.7	6938	34.69	46.69
3 LL	7.1	2.0	5	0.03	0.03
4 LR	335.4	1.1	7384	36.92	49.69



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 03 15:13:21 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040402 Sample: 0Gy 24h 50/50  
 Date: 3-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 10178 50.89%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 324,120  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	12.5	341.2	642	3.21	6.31
2 UR	89.2	173.7	8042	40.21	79.01
3 LL	0.0	0.0	0	0.00	0.00
4 LR	240.0	1.2	1494	7.47	14.68



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 03 15:15:56 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040402 Sample: (5Gy 24h 50/50)  
 Date: 3-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 8234 41.17%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 324,120  

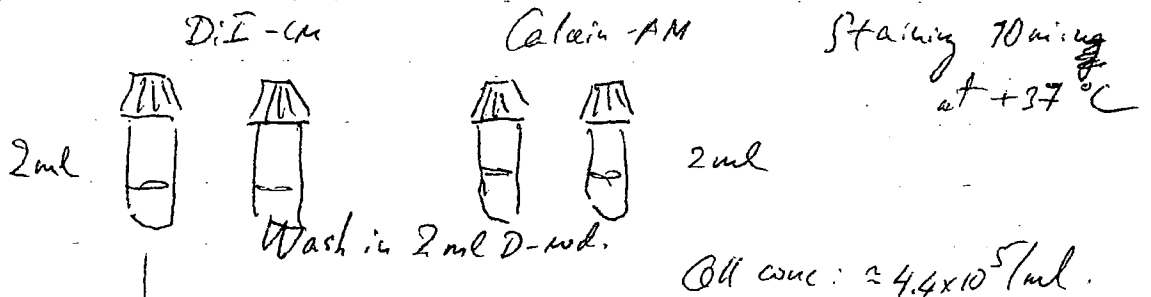
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	12.9	369.8	481	2.41	5.84
2 UR	86.6	167.8	6148	30.74	74.67
3 LL	0.0	0.0	0	0.00	0.00
4 LR	274.7	1.2	1605	8.03	19.49

APRIL 4, 2002

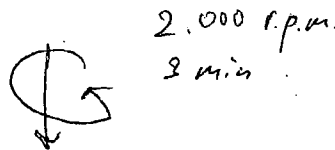
CELL-TO-CELL COMMUNICATION (50/50)  
IRRADIATION (5 Gy) OF 50% POPULATION

One 10ml dish

(27p) WB ( $\approx 90\%$  CONFLUENCY)



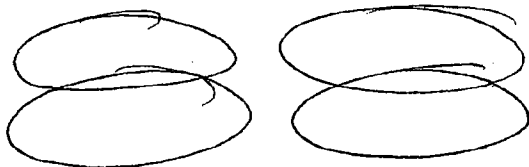
IRRADIATION (Time: 10:15)  
5 Gy.



Wash in 5ml D-medium  
Resuspend in 2ml D-medium

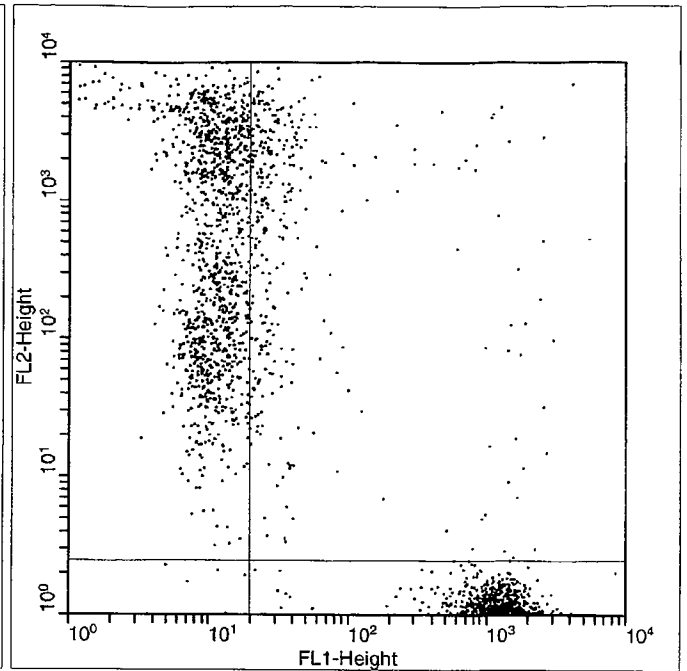
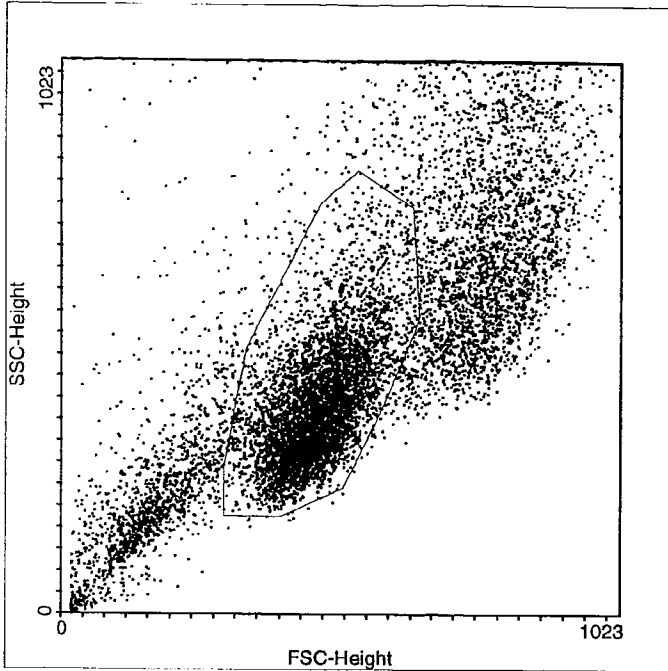
Plating into 5ml dishes (1ml + 1ml)  
IRRADIATION

3h  
6h



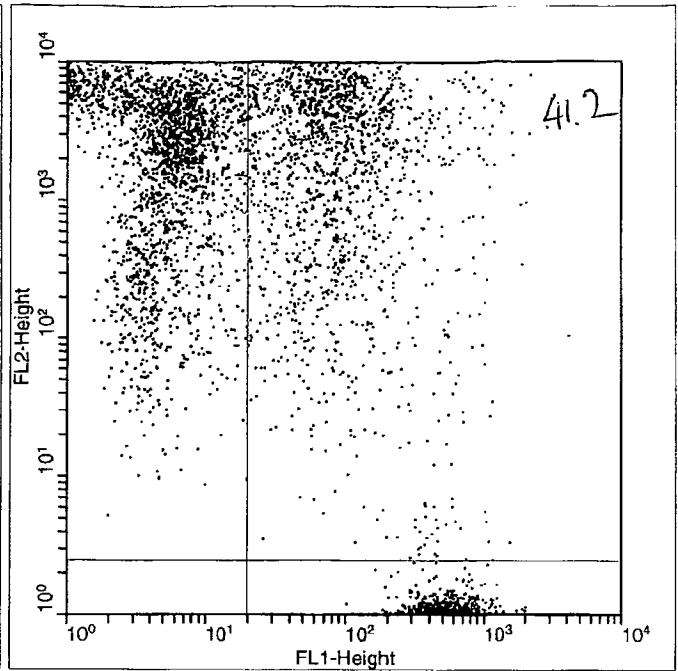
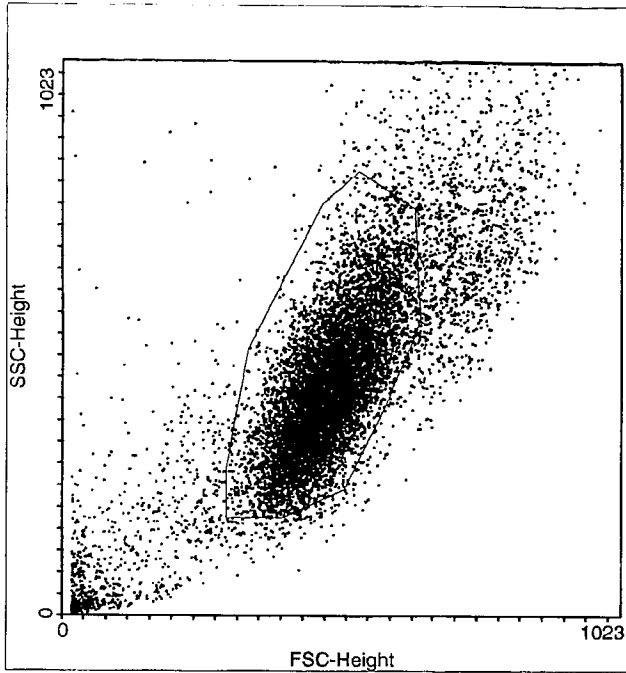
Plating time: ≈ 11:00

Good density.



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 05 11:28:08 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040502 Sample: WB mixture 0Gy Oh  
 Date: 5-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 9679 48.40%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 332,100  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	10.2	482.2	2174	10.87	22.46
2 UR	44.9	465.2	667	3.34	6.89
3 LL	11.2	1.3	23	0.12	0.24
4 LR	1674.0	1.0	6815	34.08	70.41



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 05 11:27:45 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040502 Sample: WB 0Gy 3h 50/50  
 Date: 5-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 15205 76.03%  
 System: Log Parameter Means: Geometric

Quad Stats

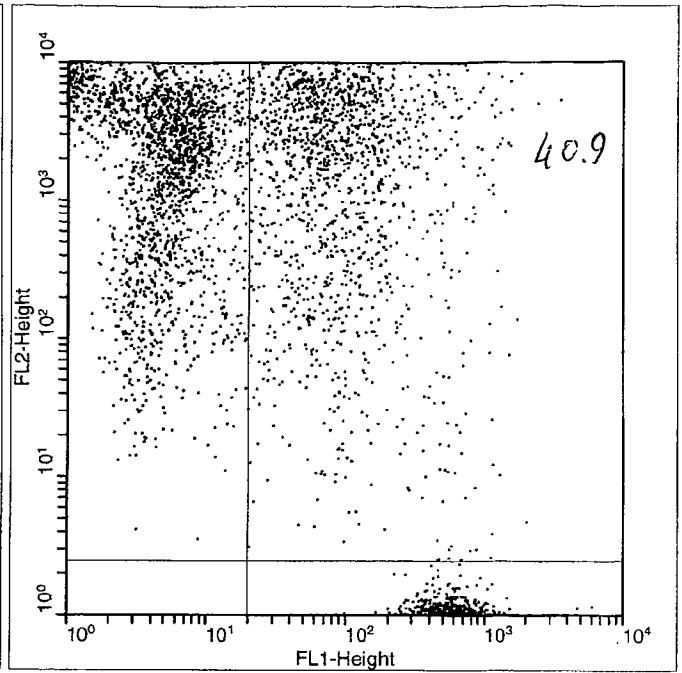
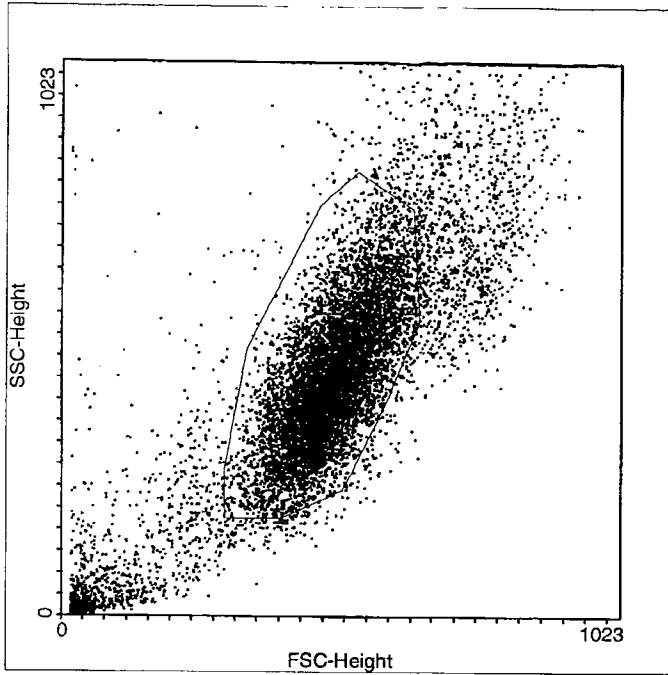
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 332,100

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.2	1939.7	4509	22.55	29.65
2 UR	94.2	1324.4	3158	15.79	20.77
3 LL	12.2	1.1	4	0.02	0.03
4 LR	627.0	1.0	7534	37.67	49.55

50.42

41.2



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 05 11:27:12 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040502 Sample: WB 5Gy 3h 50150  
 Date: 5-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14818 74.09%  
 System: Log Parameter Means: Geometric  
 Quad Stats

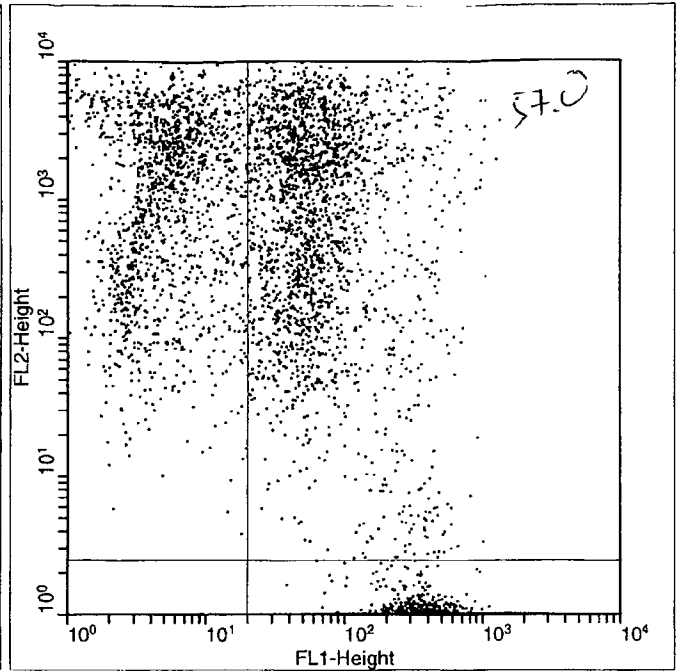
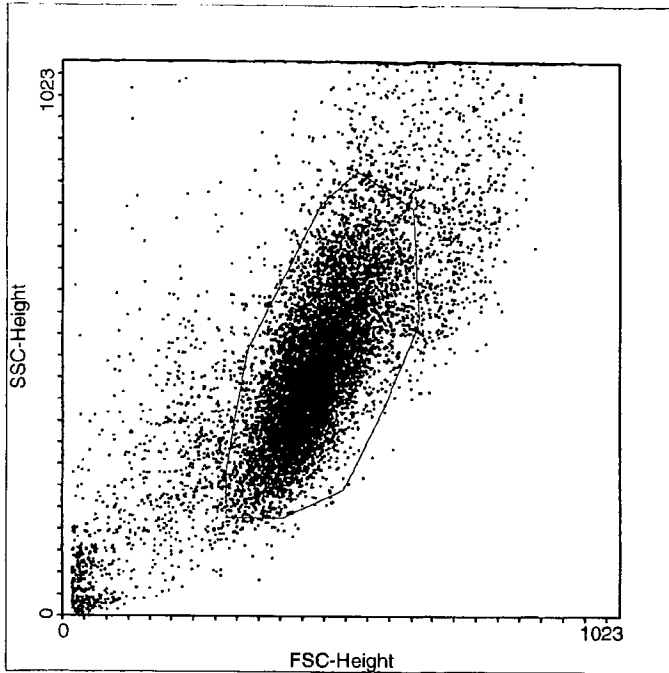
FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 332,100

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.1	1771.0	3892	19.46	26.27
2 UR	96.0	1158.6	2698	13.49	18.21
3 LL	6.1	1.1	4	0.02	0.03
4 LR	634.1	1.0	8224	41.12	55.50

44.48

40.9





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 05 11:25:55 2002

Gates: R1

Project: Experiment:

File: 040502 Sample: WB 0Gy 6h 50/50

Date: 5-Apr-2 Parameters: 7

Total Events 20000 Gated Events 15940 79.70%

System: Log Parameter Means: Geometric

Quad Stats

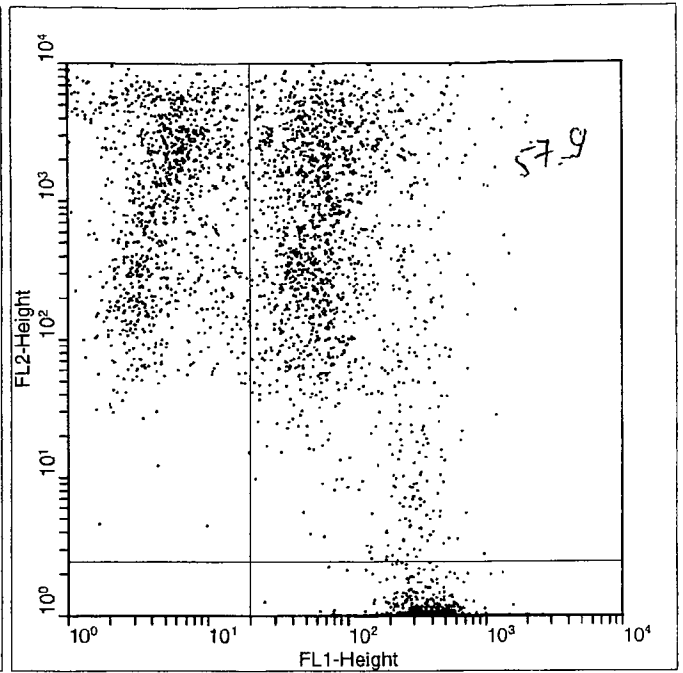
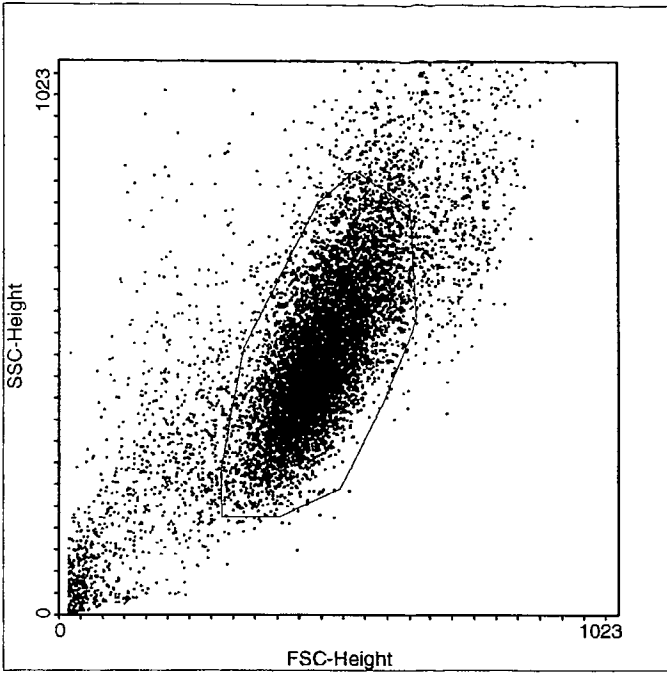
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 332,100

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.7	1110.6	3095	15.48	19.42
2 UR	70.7	639.2	4110	20.55	<u>25.78</u>
3 LL	7.9	1.4	4	0.02	0.03
4 LR	380.4	1.0	8731	43.66	<u>54.77</u>

45.2

57.0



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 05 11:24:53 2002

Gates: R1

Project: Experiment:

File: 040502 Sample: WB 5Gy 6h 50/50

Date: 5-Apr-2 Parameters: 7

Total Events 20000 Gated Events 15756 78.78%

System: Log Parameter Means: Geometric

Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 332,100

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.5	1136.0	2386	11.93	15.14
2 UR	81.4	514.4	3280	16.40	20.82
3 LL	0.0	0.0	0	0.00	0.00
4 LR	386.3	1.0	10090	50.45	64.04

35.96

579

APRIL 5, 2002

CELL-TO-CELL COMMUNICATION (50/50) (staining)  
IRRADIATION (5 Gy) OF 50% POPULATION

10 ml dishes

(27p) (100% confluency)

IRRADIATION TIME: 8:30

Staining (see protocol of APRIL 4, 2002) 12:30

Plating : 14:00

Harvesting: at 5h and 24h.

APRIL 7, 2002

CELL-TO-CELL COMMUNICATION (ISO/staining)  
IRRADIATION (5 Gy) OF 50% POPULATION

PROTOCOL #1

5 ml dishes (#3)

28p (100% confluent)

1) STAINING (15 min. at +37°C)

1) CA

2) DiI

5 Gy

3) DiI

2) WASHING WITH PBS (2x)

3) REFEEDING WITH D-MEDIUM FOR 10 min. at +37°C

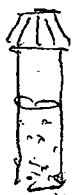
4) IRRADIATION WITH 5 Gy (<sup>137</sup>Cs  $\gamma$ -rays) Time: 11:00

5) TRYPsinIZATION (0.5 ml) + D-MEDIUM (2 ml)

6) HARVESTING INTO THREE PLASTIC TUBES



CA



DiI



DiI (5Gy)

7) WASHING WITH D-MEDIUM (3 ml)

8) PLATING (CO-CULTURING) 0.5 ml ( $\approx 1 \times 10^6$  cells)

3L - CA + DiI

CA + DiI

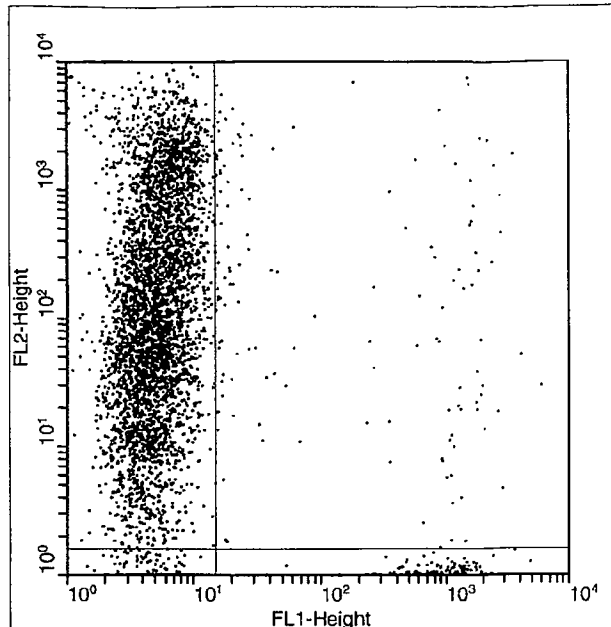
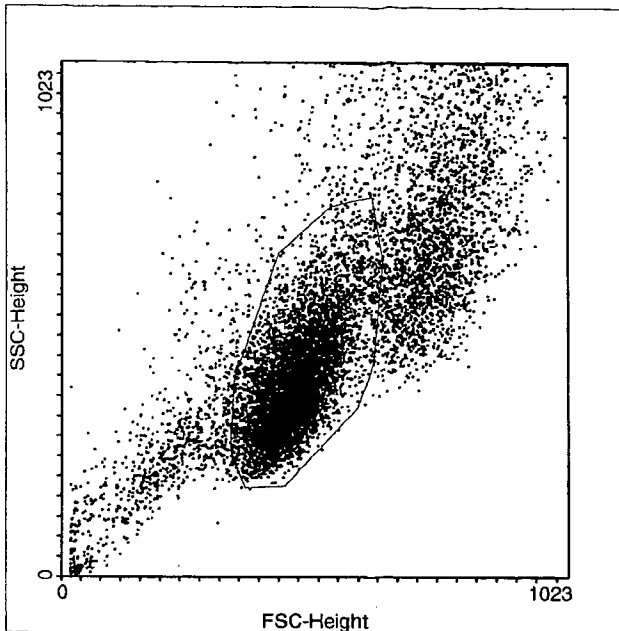
6L - CA + DiI

CA + DiI

Time: 11:45

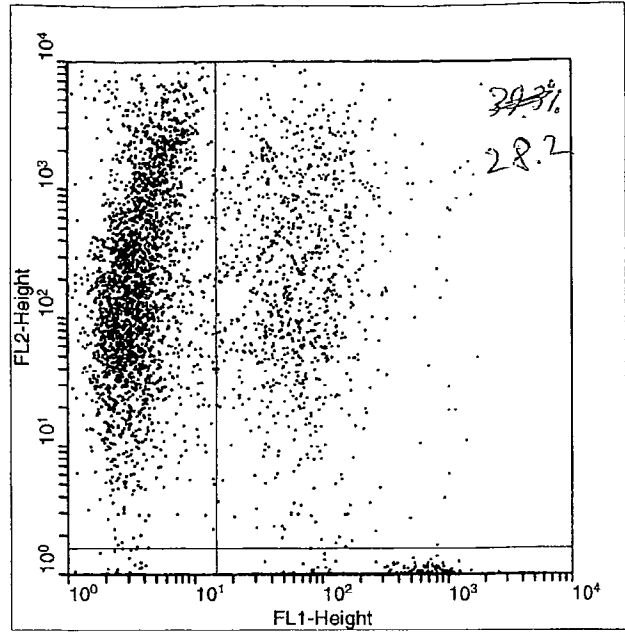
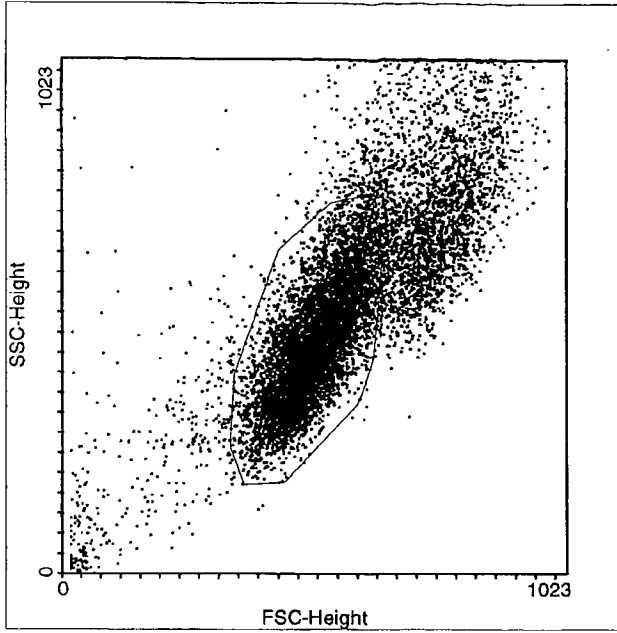
Time: 14:45 } HARVESTING  
Time: 17:45 } FOR FCM

PROTOCOL # 1



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 08 14:07:47 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040802 Sample: Mixture (0Gy 0h)  
 Date: 8-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 11844 59.22%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 300,52

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.0	134.1	7739	38.70	65.34
2 UR	128.4	163.2	280	1.40	2.36
3 LL	4.8	1.1	322	1.61	2.72
4 LR	1463.9	1.0	3503	17.52	29.58



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 08 14:10:18 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040802 Sample: OGy 3h  
 Date: 8-Apr-2 Parameters:  
 Total Events 20000 Gated Events 12868 64.34%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 300,52

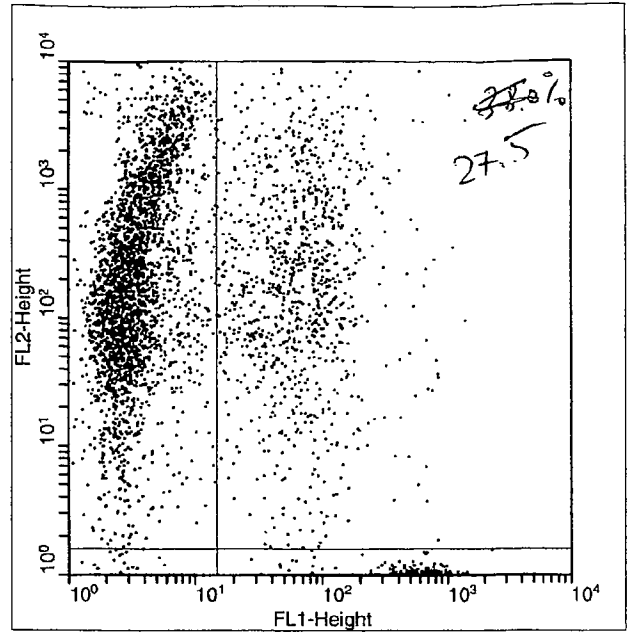
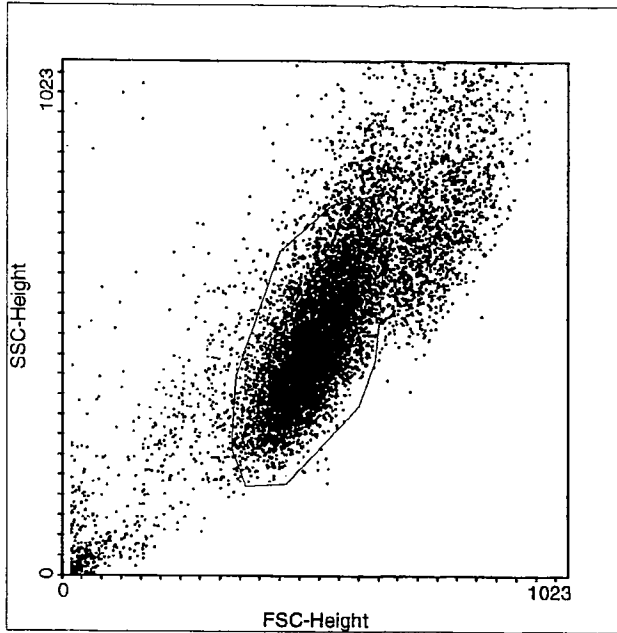
53.73

P/L  
CA

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.7	205.7	4965	24.83	38.58
2 UR	71.0	204.3	1949	9.74	15.15
3 LL	3.3	1.1	91	0.46	0.71
4 LR	654.2	1.0	5863	29.32	45.56

28.2 } 53.7%

PROTOCOL # 1



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 08 14:10:47 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040802 Sample: 5Gy 3h  
 Date: 8-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14121 70.61%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 300,52

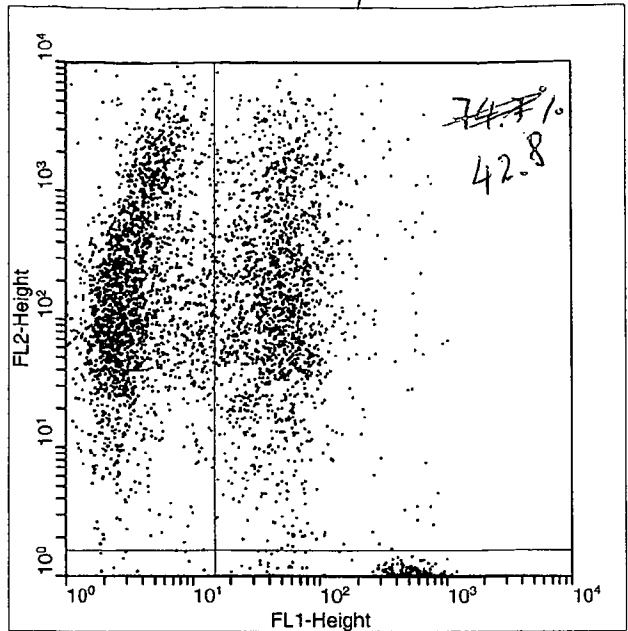
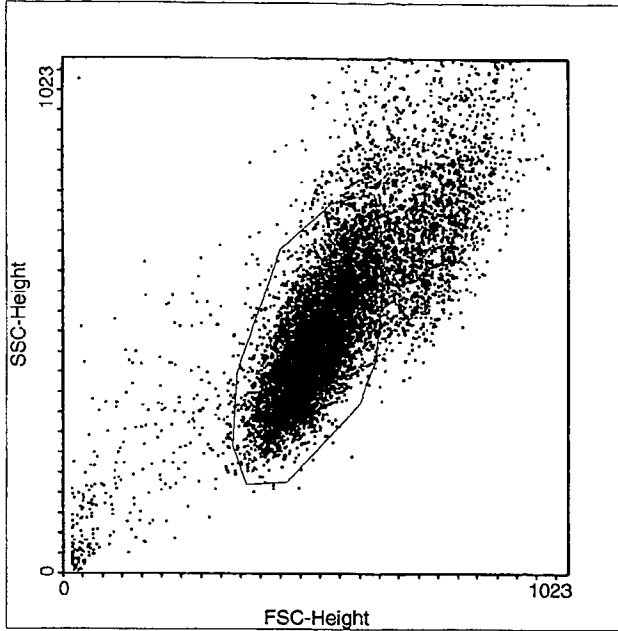
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.5	197.2	5136	25.68	36.37
2 UR	67.2	195.6	1953	9.77	13.83
3 LL	3.7	1.1	118	0.59	0.84
4 LR	616.4	1.0	6914	34.57	48.96

27.5  
 CA

50.2

27.5

PROTOCOL #1



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 08 14:11:15 2002  
 Gates: R1  
 Project: Experiment  
 File: 040802 Sample: (OGy 6h)  
 Date: 8-Apr-2 Parameters:  
 Total Events 20000 Gated Events 14590 72.95%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 300,52

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.6	169.3	4559	22.80	31.25
2 UR	50.1	158.0	3405	17.03	23.34
3 LL	4.1	1.1	66	0.33	0.45
4 LR	437.1	1.0	6560	32.80	44.96

CA  
 P.L.

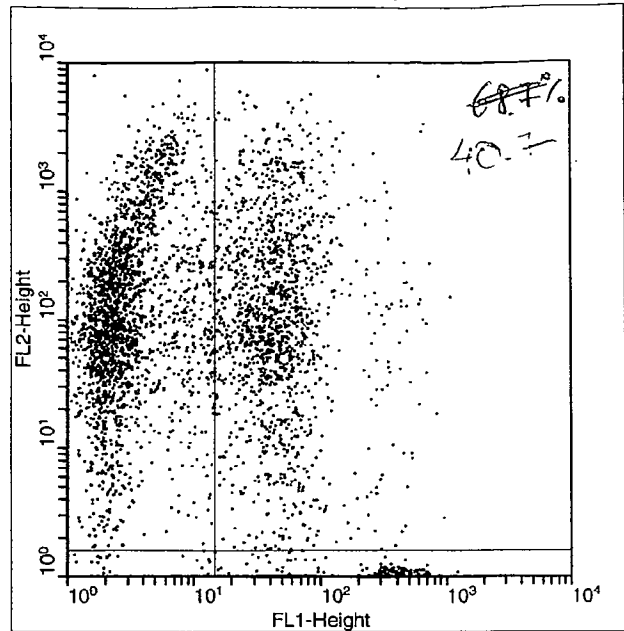
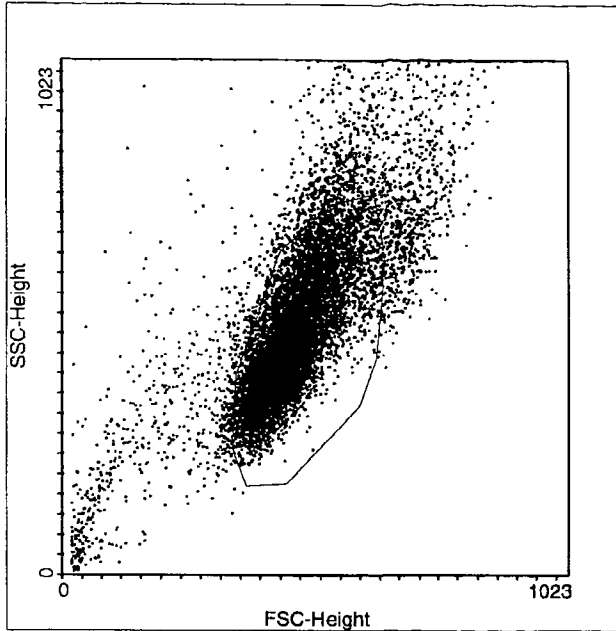
Handwritten calculations:  

$$\begin{array}{r} 31.25 \\ + 23.34 \\ \hline 54.59 \\ \times 23.34 \\ \hline 1271.115 \\ + 1001.000 \\ \hline 2272.115 \end{array}$$
  

$$\frac{2272.115}{54.59} = 41.82$$
  
 42.8



PROTOCOL #1



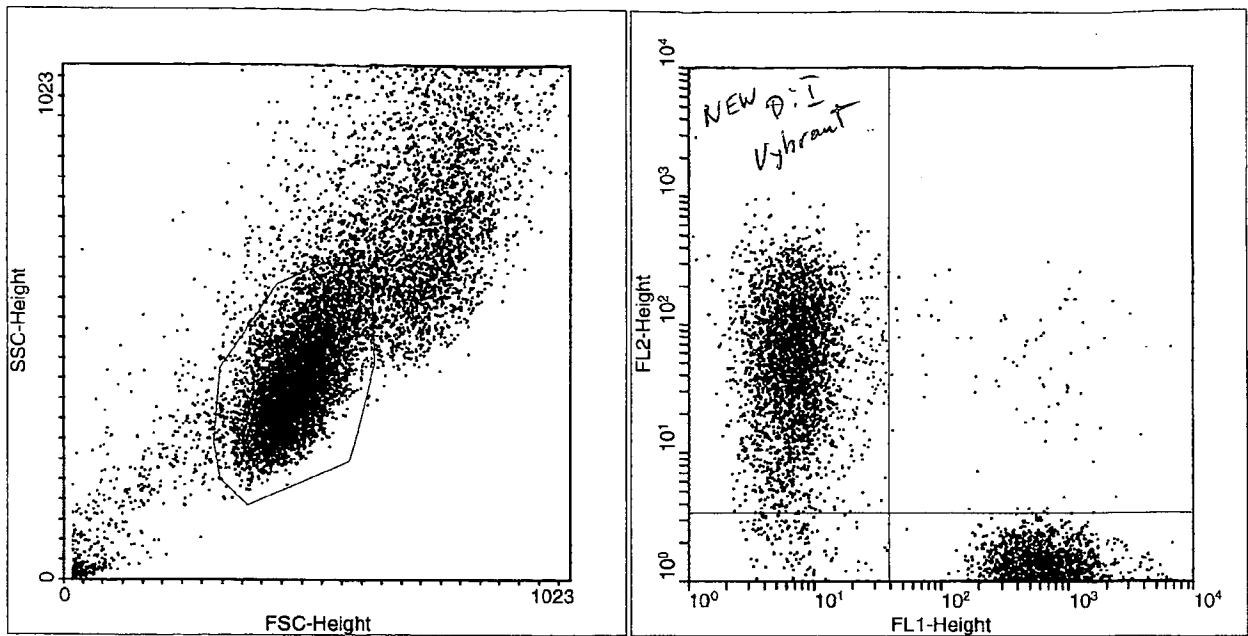
Multiple Document Interface for Flow Cytometry  
WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
Mon Apr 08 14:11:33 2002  
Gates: R1  
Project: Experiment  
File: 040802 Sample: 5Gy 6h  
Date: 8-Apr-2 Parameters:  
Total Events 20000 Gated Events 15643 78.21%  
System: Log Parameter Means: Geometric  
Quad Stats  
FL1-Height(Log) vs FL2-Height(Log)  
Quadrant x,y: 300,52

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.1	119.9	4110	20.55	26.27
2 UR	48.1	111.0	2825	14.13	18.06
3 LL	4.0	1.1	92	0.46	0.59
4 LR	362.7	1.0	8616	43.08	55.08

44.33

40.7

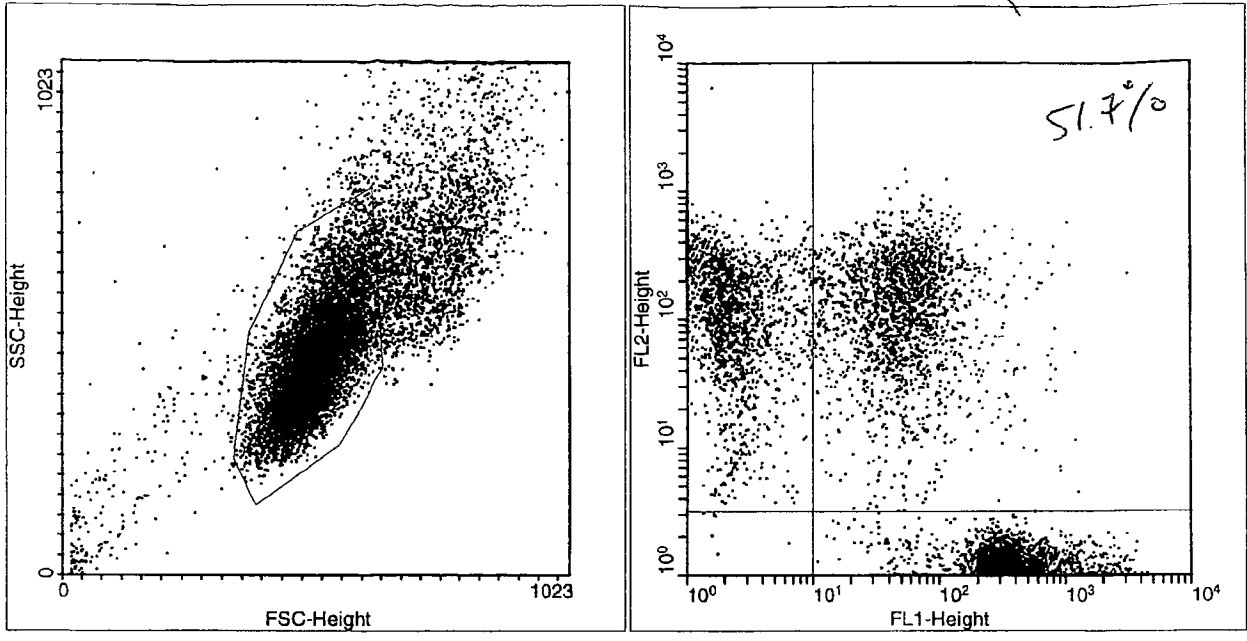
Protocol #12



Multiple Document Interface for Flow Cytometry  
WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
Mon Apr 08 14:25:09 2002  
Gates: R1  
Project: Experiment:  
File: 040802 Sample: Mixture (0Gy 0h)  
Date: 8-Apr-2 Parameters: 7  
Total Events 20000 Gated Events 10618 53.09%  
System: Log Parameter Means: Geometric  
Quad Stats  
FL1-Height (Log) vs FL2-Height (Log)  
Quadrant x,y: 408,136

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	6.9	54.2	5997	29.99	56.48
2 UR	416.0	42.4	168	0.84	1.58
3 LL	7.0	2.0	236	1.18	2.22
4 LR	747.4	1.3	4217	21.09	39.72

PROTOCOL#2



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 08 14:43:52 2002  
 Gates: R1

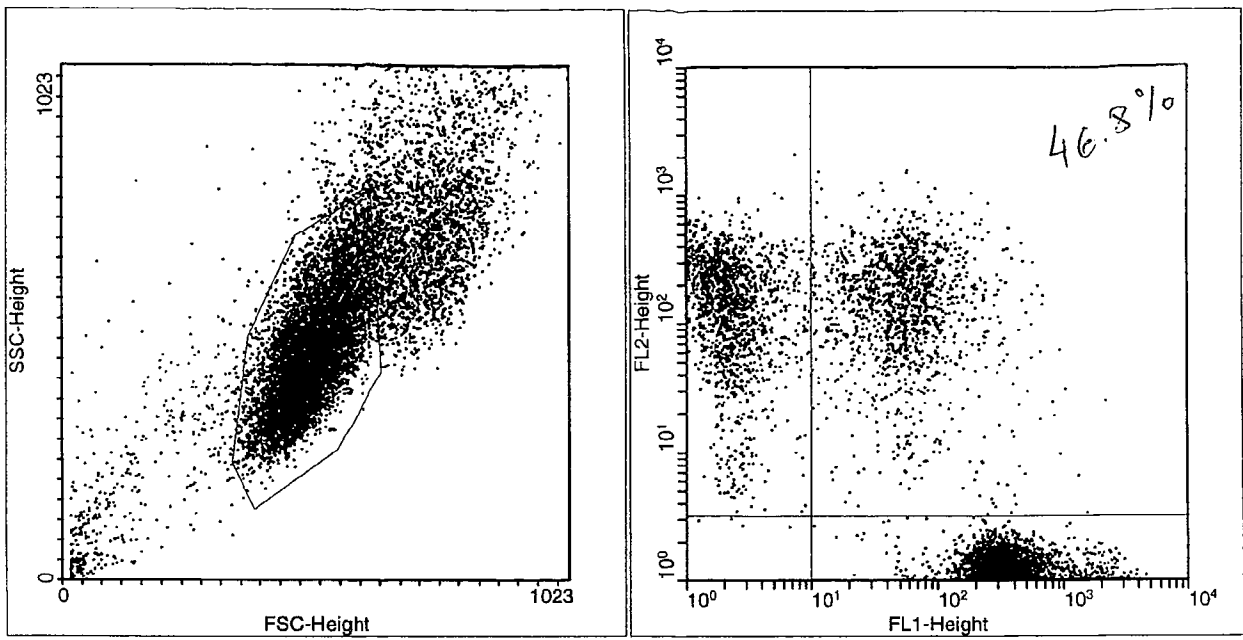
Project: Experiment:  
 File: 040802 Sample: Ogy 5h  
 Date: 8-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14579 72.89%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 256,128

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.3	107.7	3383	16.92	23.20
2 UR	51.3	109.6	3618	18.09	24.82
3 LL	2.3	2.4	10	0.05	0.07
4 LR	422.2	1.1	7568	37.84	51.91

51.67  
 48.02%

PROTOCOL #2



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 08 14:43:19 2002  
 Gates: R1  
 Project: Experiment:  
 File: 040802 Sample: 5Gy 5h  
 Date: 8-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13709 68.54%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 256,128

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.2	134.8	2936	14.68	21.42
2 UR	55.1	125.6	2583	12.92	18.84
3 LL	2.7	2.8	4	0.02	0.03
4 LR	371.0	1.1	8186	40.93	59.71

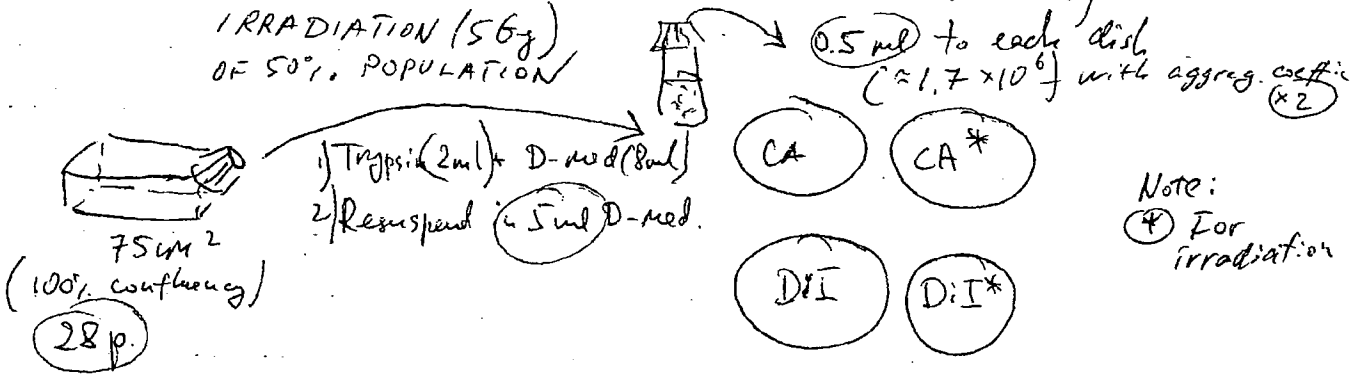
46.8  
 40.26

PROTOCOL  
FOR "DYE TRANSFER"

April 9, 2002

CELL-TO-CELL COMMUNICATION (50/50) staining)

IRRADIATION (56g)  
OF 50% POPULATION



Note:  
⊕ For irradiation



April 11, 2002

100% Confluency

⊕ STAINING (DYES WERE ADDED DIRECTLY TO THE MEDIUM)

For example, DiI - 5  $\mu$ l ; Calcein-AM - 40  $\mu$ l.  
15 min at +37°C

⊕ WASHING WITH PBS (2<sup>x</sup>)

⊕ REFEEDING WITH D-MEDIUM FOR 10 min at +37°C

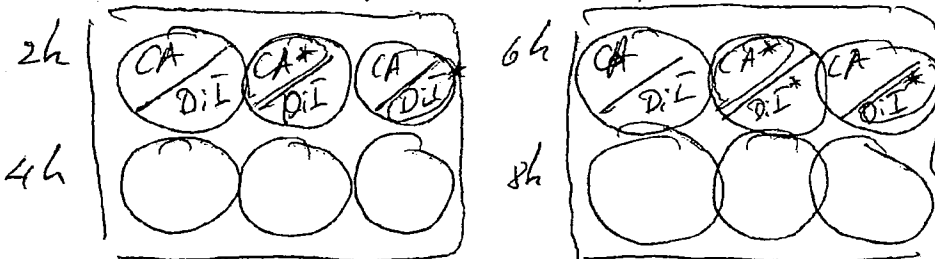
⊕ IRRADIATION WITH TWO SOURCES (688.65 Rad/min) of <sup>137</sup>Cs,  
i.e.  $\approx 96g$ .  
Time: 9:10

⊕ INCUBATION AT +37°C FOR 10 min

⊕ TRYPSINIZATION (1.0ml) + D-MEDIUM (2ml)

⊕ HARVESTING INTO ~~THREE~~ <sup>FOUR</sup> PLASTIC TUBES

⊕ PLATING 0.2ml of Time: 10:10  
 $\approx 1.9 \times 10^5$  cells of each dye ( $\Sigma = 3.8 \times 10^5$  per ~~well~~)  $\xrightarrow{\text{Aggreg. coeff.} \approx 2}$   $\approx 7.6 \times 10^5$  cells



COULTER  
COUNTS

↓ see over

Coulter counts: 2466  
 2397 }  $2448 \times 400 \approx 9.8 \times 10^5$  cells/ml  
 2480

(26) 12:10

in 20 ml ISOTONE

CA/DiI 221 }  
 234 }  $244 \times 400 = 9.8 \times 10^4$  cell/ml  
 277 }  $\times 2.5 = 24.5 \times 10^4$  cells

\* CA/DiI 221 }  
 184 }  $207 \times 400 = 8.3 \times 10^4$  cells/ml  
 217 }  $\times 2.5 = 20.8 \times 10^4$  cells

CA/DiI\* 244 }  
 258 }  $255 \times 400 = 10.2 \times 10^4$  cells/ml  
 262 }  $\times 2.5 = 25.5 \times 10^4$  cells

46 14:10

in 10 ml isotone

CA/DiI

$$\left. \begin{array}{l} 737 \\ 701 \end{array} \right\} \frac{719 \times 400}{2} = 1.44 \times 10^5 \text{ cells/ml}$$

$$\times 2.5 = 36 \times 10^4 \text{ cells/ml}$$

CA\*/DiI

$$\left. \begin{array}{l} 986 \\ 1017 \\ 965 \end{array} \right\} \frac{989 \times 400}{2} = 1.98 \times 10^5 \text{ cells/ml}$$

$$\times 2.5 = 49 \times 10^4 \text{ cells/ml}$$

CA/DiI\*

$$\left. \begin{array}{l} 989 \\ 1025 \end{array} \right\} \frac{1007 \times 400}{2} = 2.01 \times 10^5 \text{ cells/ml}$$

$$\times 2.5 = 50.3 \times 10^4 \text{ cells/ml}$$

64 16:10

in 20 ml isotone (again)

CA/DiI

$$\left. \begin{array}{l} 561 \\ 576 \\ \del{620} \\ 540 \end{array} \right\} 559 \times 400 = 2.24 \times 10^5 \text{ cells/ml}$$

$$\times 2.5 = 56 \times 10^4 \text{ cells/ml}$$

Ca\*/DiI

$$\left. \begin{array}{l} 601 \\ 558 \\ 538 \end{array} \right\} 566 \times 400 = 2.26 \times 10^5 \text{ cells/ml}$$

$$\times 2.5 = 56.5 \times 10^4 \text{ cells/ml}$$

CA/DiI\*

$$\left. \begin{array}{l} 577 \\ 550 \\ 524 \end{array} \right\} 550 \times 400 = 2.20 \times 10^5 \text{ cells/ml}$$

$$\times 2.5 = 55 \times 10^4 \text{ cells/ml}$$



8h

18:10 in 20 ml isotope

CA/D: I

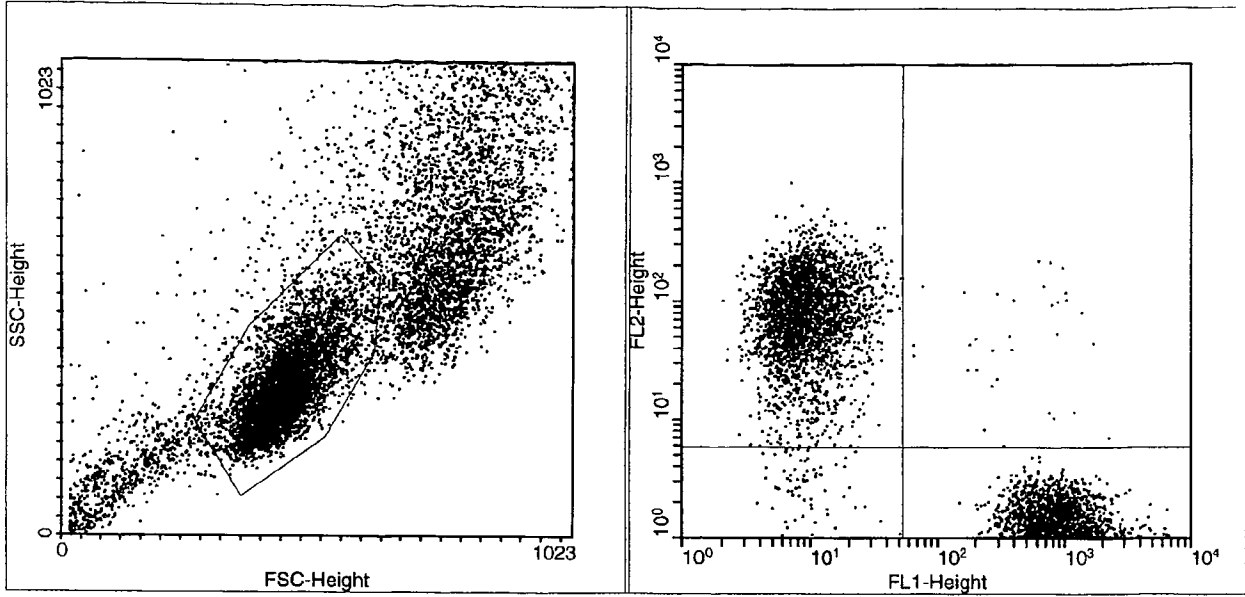
557 }  
 565 }  $557 \times 400 = 2.22 \times 10^5$  cells/ml  
~~518~~ }  $\times 2.5 = 55.7 \times 10^4$  cells  
 550 }

CA\*/D: I

554 }  
 547 } 557 — " —  $55.7 \times 10^4$  cells  
 570 }

CA/D: I\*

619 }  
 604 }  $612 \times 400 = 2.45 \times 10^5$  cells/ml  
 612 }  $\times 2.5 = 61.2 \times 10^4$  cells



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:17:36 2002

Gates: R1

Project: Experiment: Control 0h

File: 041202 Sample: Control 0h  
 Date: 12-Apr-2 Parameters: 7

Total Events 20000 Gated Events 10222 51.11%

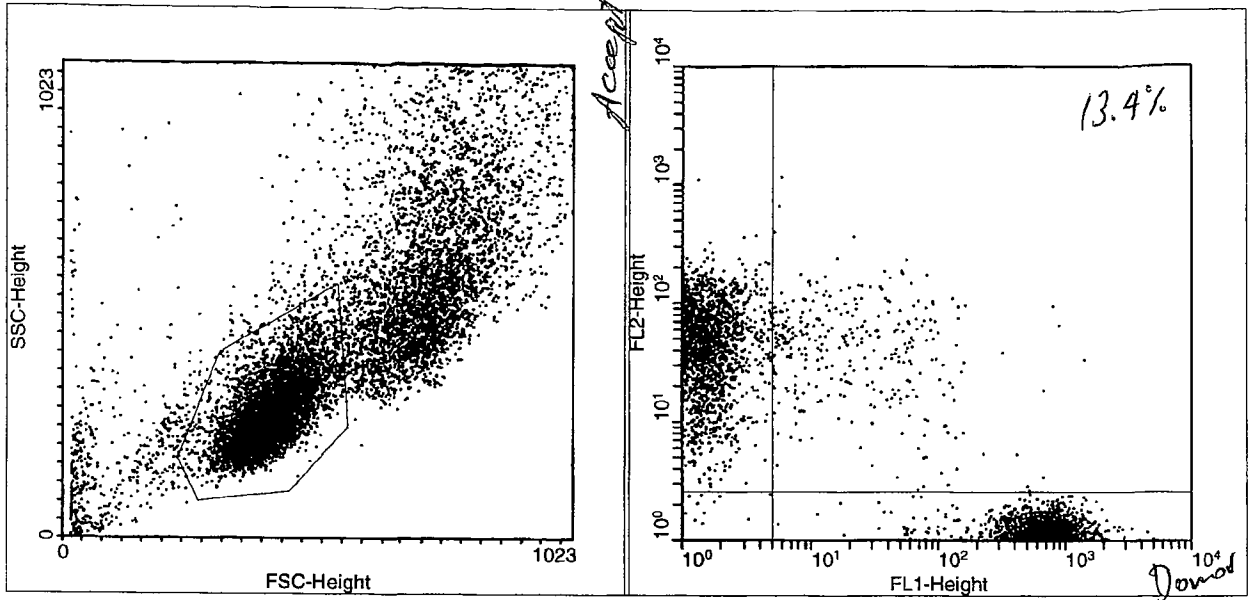
System: Log Parameter Means: Geometric

Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 444,196

Quad	X-Mean	Y-Mean	Events	%Total	%Gated	
1 UL	9.2	76.4	5560	27.80	54.39	- D.I
2 UR	335.7	53.1	61	0.31	0.60	
3 LL	9.4	2.5	142	0.71	1.39	
4 LR	940.6	1.3	4459	22.30	43.62	- CA

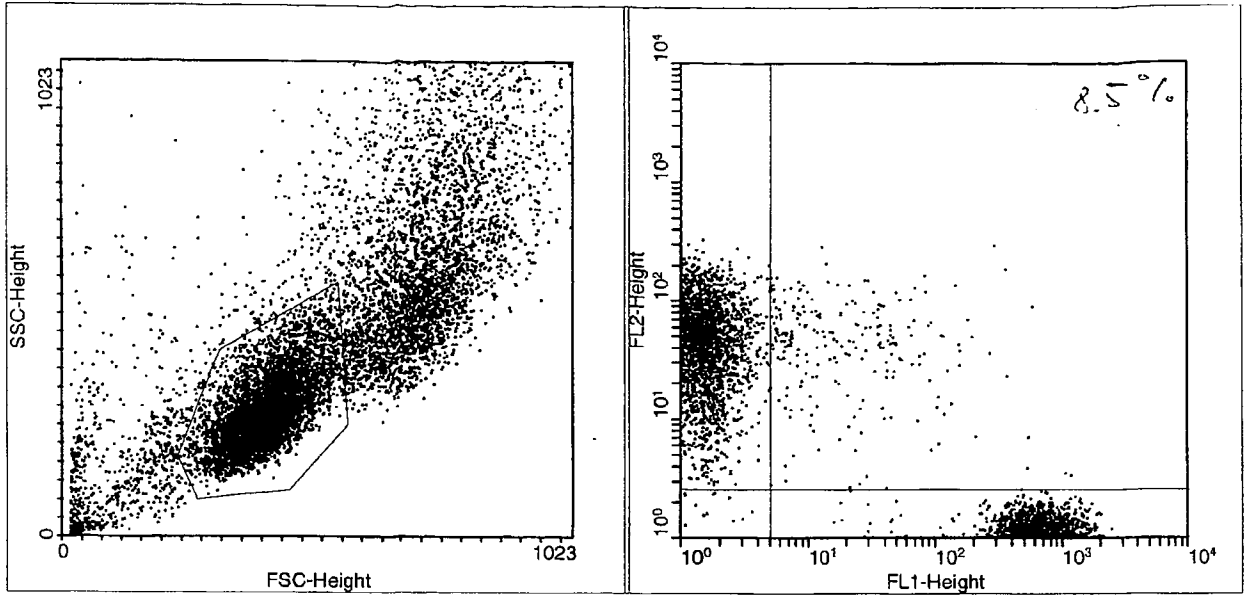


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:29:37 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041202 Sample: CA/DiI 2h 9 Gy  
 Date: 12-Apr-2 Parameters:  
 Total Events 20000 Gated Events 10322 51.61%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	46.0	4124	20.62	39.95
2 UR	23.4	37.0	640	3.20	6.20
3 LL	1.5	2.0	24	0.12	0.23
4 LR	702.0	1.1	5534	27.67	53.61

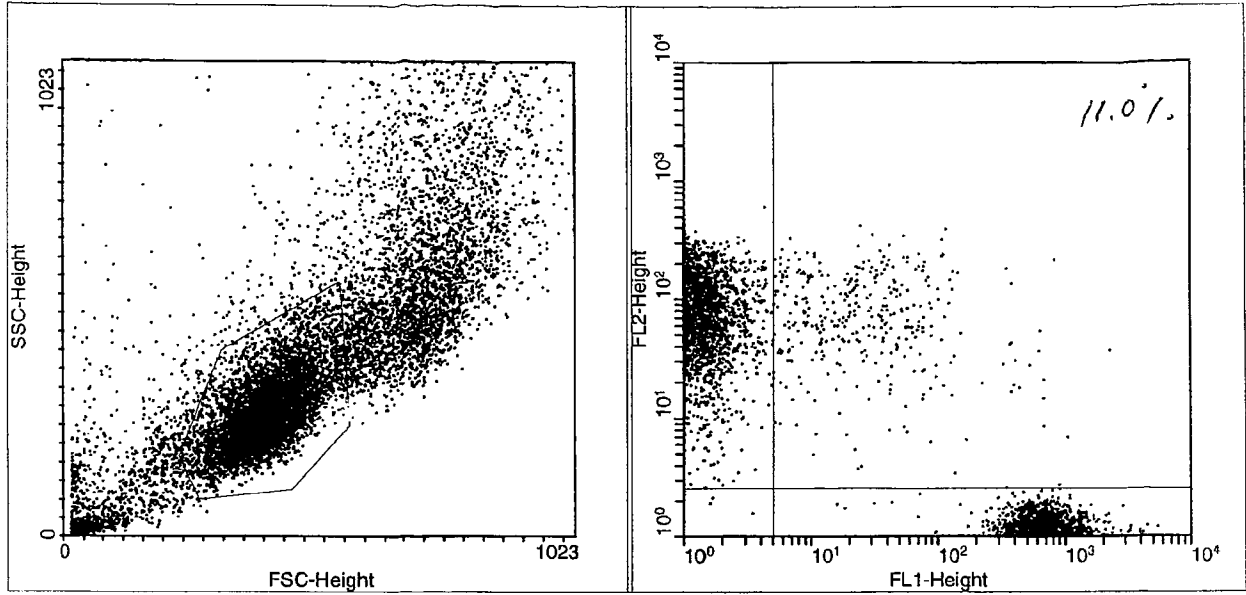
46.15 → 13.4%



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:27:57 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041202 Sample: CA<sup>9</sup>/DiI 2h 9Gy  
 Date: 12-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 10248 51.24%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	45.1	4950	24.75	48.30
2 UR	22.2	38.3	461	2.31	4.50
3 LL	1.8	1.9	28	0.14	0.27
4 LR	706.5	1.1	4809	24.05	46.93

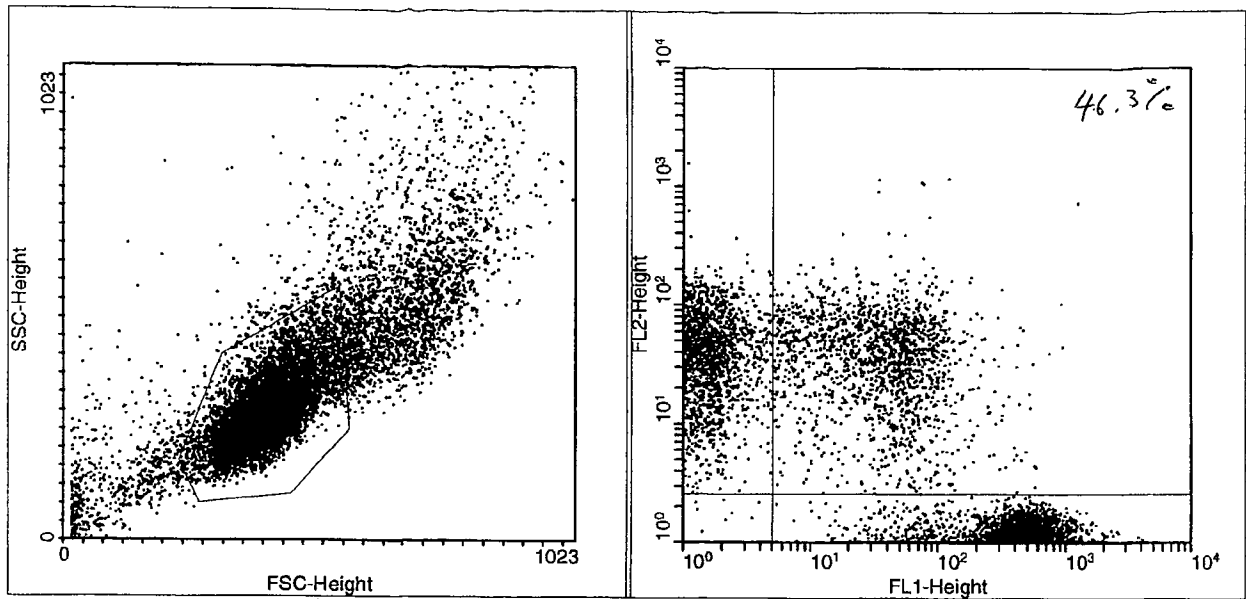
52.8 → 8.5%



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:35:42 2002  
 Gates: R1  
 Project: Experiment;  
 File: 041202 Sample: CA/DiI\* 2h 9Gy  
 Date: 12-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 10795 53.97%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.3	77.6	5166	25.83	47.86
2 UR	29.4	63.8	639	3.20	5.92
3 LL	1.6	1.9	12	0.06	0.11
4 LR	716.4	1.1	4978	24.89	46.11

53.8 → 11.0%

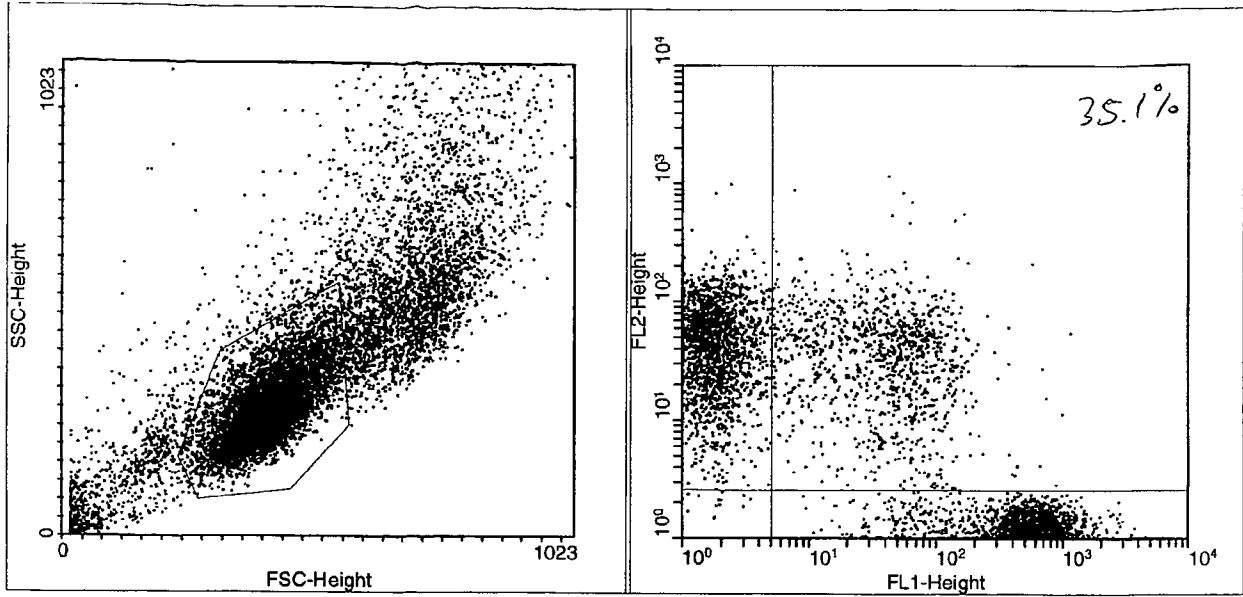


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:37:48 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041202 Sample: CA/DiI 4h 0Gy  
 Date: 12-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14009 70.04%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	39.1	3497	17.49	24.96
2 UR	34.7	31.8	3009	15.05	21.48
3 LL	1.7	1.9	19	0.10	0.14
4 LR	405.1	1.1	7484	37.42	53.42

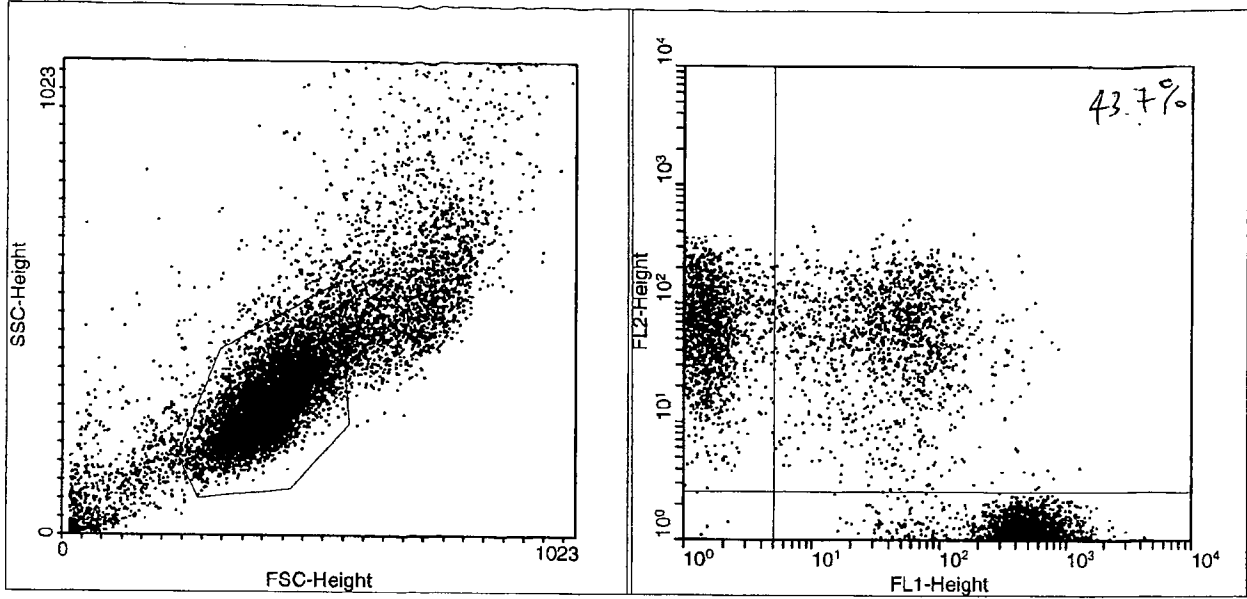
46.4 → 46.3%  
 53.42 - no clearance



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:41:40 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041202 Sample: CA\*/DiI 4h 9Gy  
 Date: 12-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 12020 60.10%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.8	40.8	3840	19.20	31.95
2 UR	35.7	31.6	2074	10.37	17.25
3 LL	1.9	1.9	24	0.12	0.20
4 LR	503.9	1.1	6082	30.41	50.60

49.2% → 35.1%  
 ↑ ?



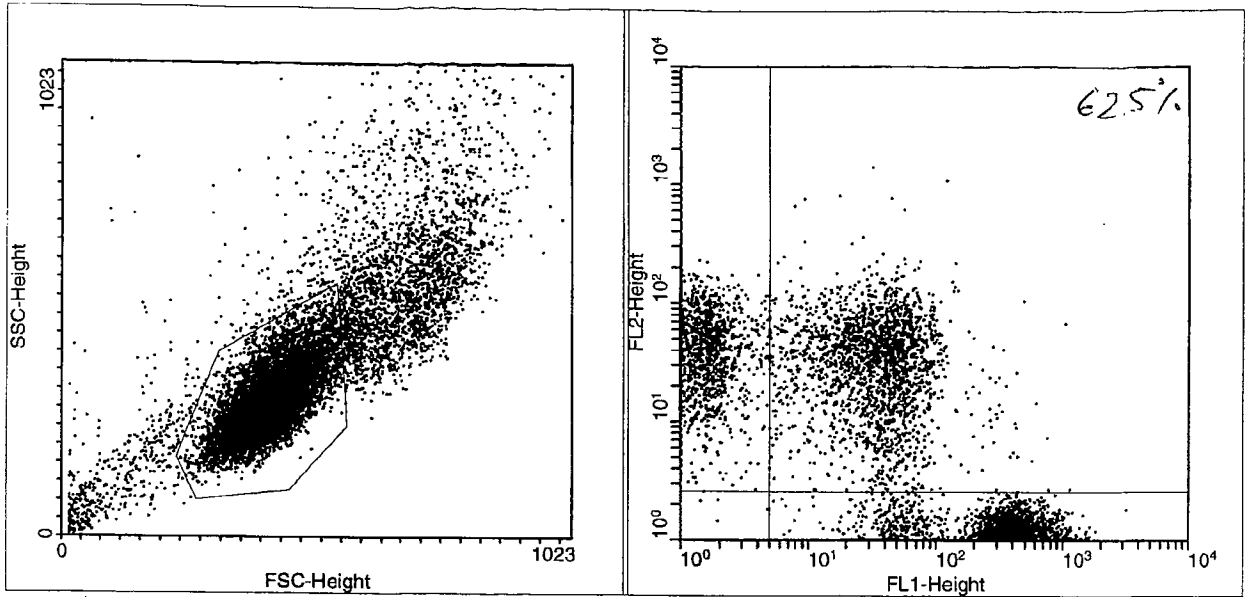
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:42:34 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041202 Sample: CA/DiI\* 4h 9Gy  
 Date: 12-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13815 69.07%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	66.9	3727	18.64	26.98
2 UR	41.8	54.4	2891	14.46	20.93
3 LL	1.5	1.8	13	0.07	0.09
4 LR	488.1	1.1	7184	35.92	52.00

47.9 → 43.7

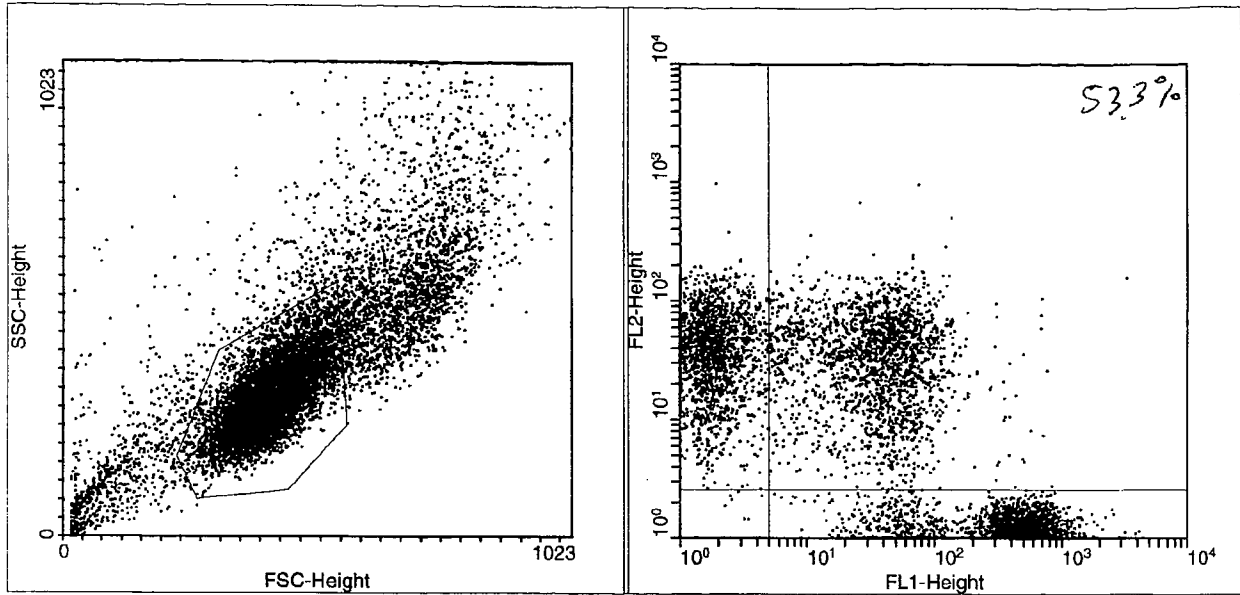




Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:46:50 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041202 Sample: CA/DiI 6h OG  
 Date: 12-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13967 69.83%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	37.5	2448	12.24	17.53
2 UR	33.7	29.1	4076	20.38	29.18
3 LL	2.2	1.7	5	0.03	0.04
4 LR	322.7	1.1	7438	37.19	53.25

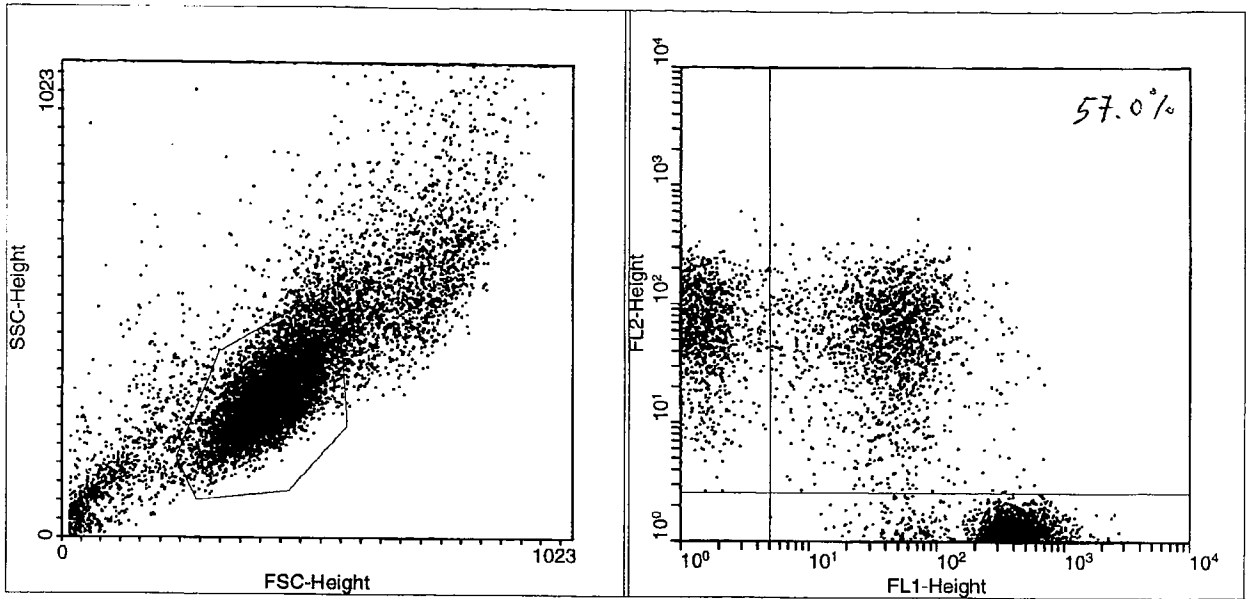
46.7 → 62.5%  
 - NO CHANGE



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:48:37 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041202 Sample: CA\*/DiI 6h 9Gy  
 Date: 12-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13499 67.50%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.8	36.2	3210	16.05	23.78
2 UR	33.6	29.8	3659	18.30	27.11
3 LL	2.7	1.9	18	0.09	0.13
4 LR	372.2	1.1	6612	33.06	48.98

50.9% → 53.3%  
 NO CHANGE

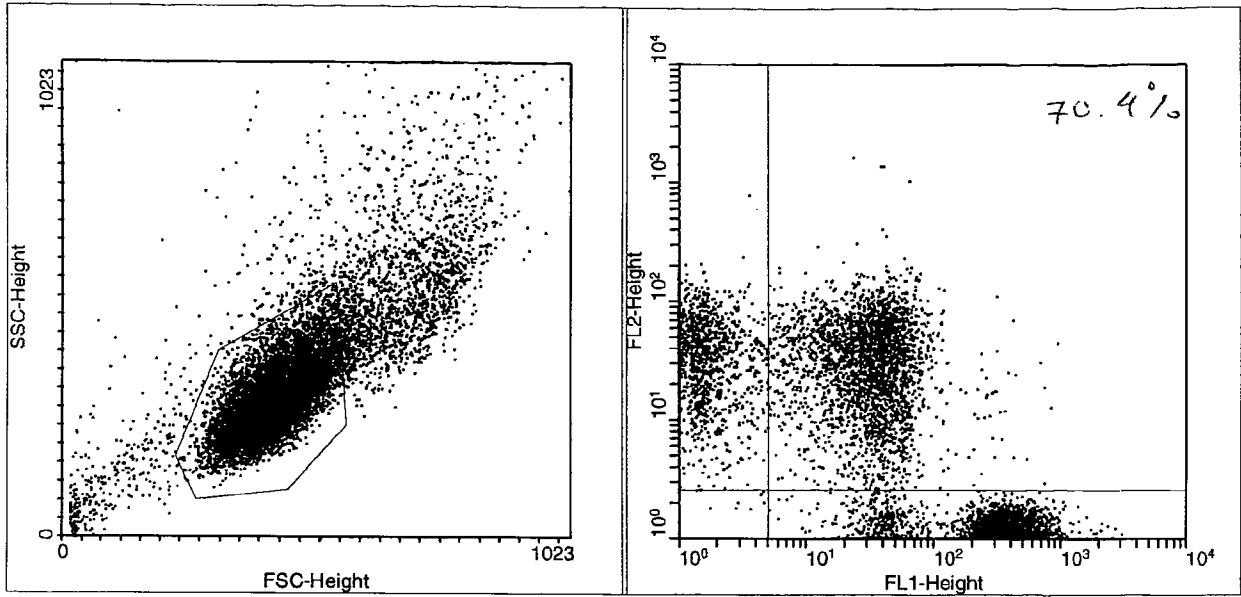


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:49:01 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041202 Sample: CA/DiI\* 6h 9Gy  
 Date: 12-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13675 68.38%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	63.9	2623	13.12	19.18
2 UR	41.5	49.1	3479	17.40	25.44
3 LL	0.0	0.0	0	0.00	0.00
4 LR	390.0	1.1	7573	37.87	55.38

44.6 → 57.0%



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:50:28 2002

Gates: R1

Project: Experiment:

File: 041202 Sample: CA/DiI 8h 0Gy

Date: 12-Apr-2 Parameters: 7

Total Events 20000 Gated Events 15414 77.07%

System: Log Parameter Means: Geometric

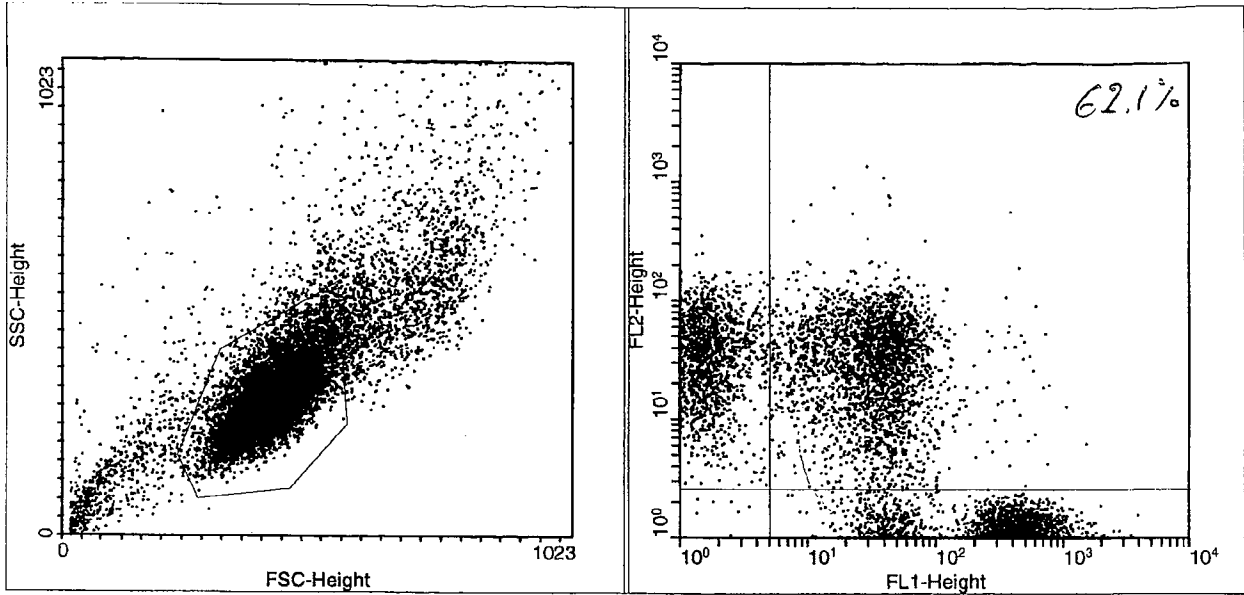
Quad Stats

FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	33.0	2203	11.02	14.29
2 UR	31.2	28.7	5216	26.08	33.84
3 LL	2.5	2.0	14	0.07	0.09
4 LR	296.4	1.1	7981	39.91	51.78

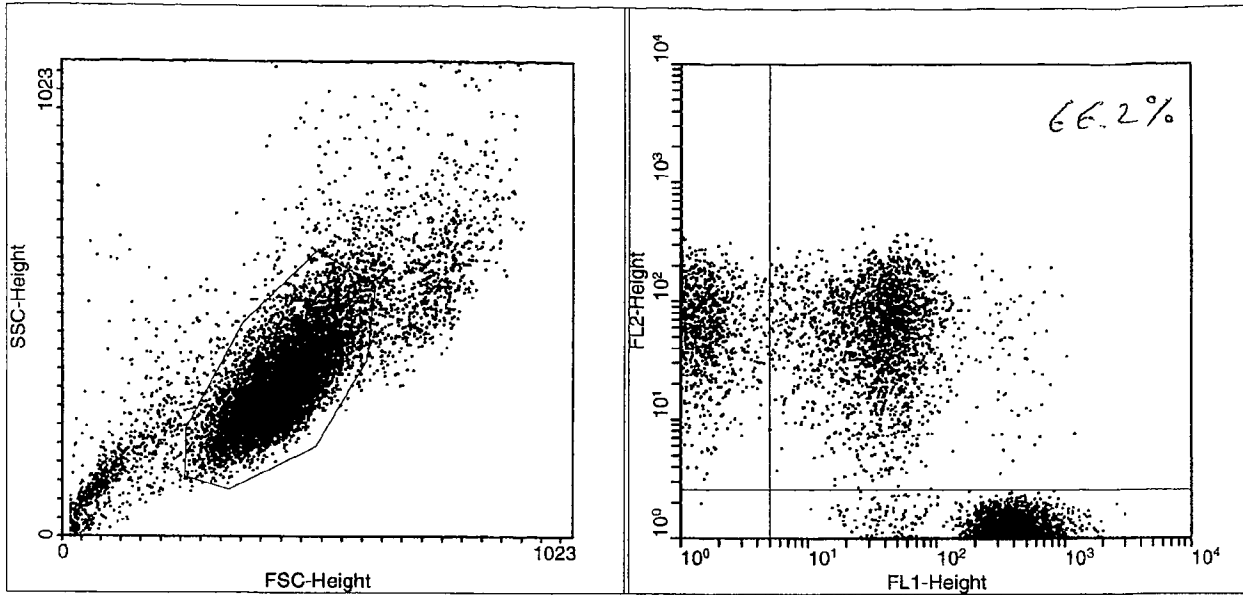
48.1 → 70.4%  
 - NO CHANGE



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 11:50:59 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041202 Sample: CA\*/DiI 8h 9Gy  
 Date: 12-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14803 74.01%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	34.9	3009	15.05	20.33
2 UR	31.6	28.3	4926	24.63	33.28
3 LL	2.1	1.8	15	0.08	0.10
4 LR	315.6	1.1	6853	34.27	46.29

53.61 → 62.1  
 NO CHANGE



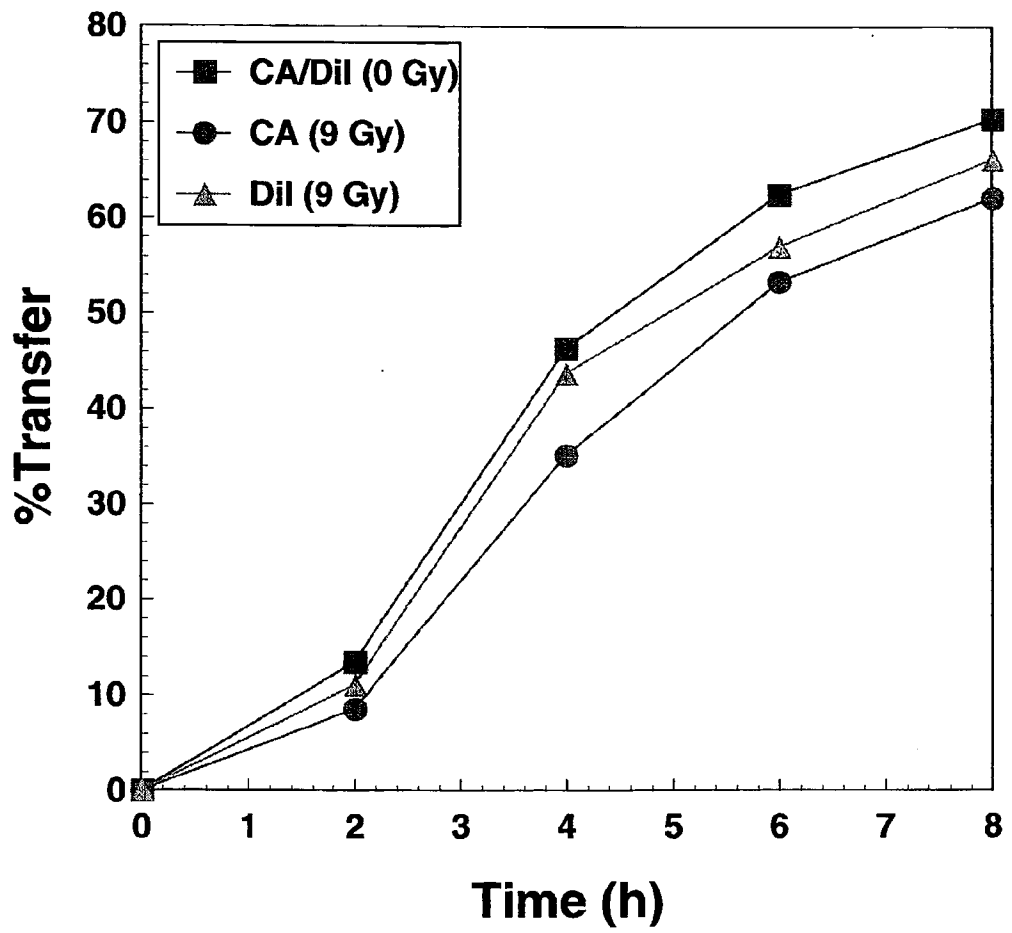
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 12 12:00:55 2002  
 Gates: R1

Project: Experiment:  
 File: 041202 Sample: CA/DiI\* 8h 9Gy  
 Date: 12-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16166 80.83%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 180,104

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	63.9	2273	11.37	14.06
2 UR	37.9	51.9	4461	22.31	27.59
3 LL	1.5	2.0	1	0.01	0.01
4 LR	364.5	1.1	9431	47.16	58.34

} 41.7 → 66.2  
 ↑



Label	A	B	C	D	E
Label	Time (h)	CA (0 Gy) DiI	CA (9 Gy) DiI	CA (9 Gy) DiI	CA (9 Gy) DiI
1	0	0	0	0	0
2	2	13.4	8.5	11	
3	4	46.3	35.1	43.7	
4	6	62.5	53.3	57	
5	8	70.4	62.1	66.2	
6					



APRIL 14, 2002

CELL-TO-CELL COMMUNICATION (50/50 - staining)  
IRRADIATION (9 Gy) OF 50% POPULATION  
(REPLICATE EXPERIMENT)


WBS - 30p

COULTER COUNTS:

IRRADIATION TIME:

















8:40

3956 }  
3972 }  $4025 \times 400 \approx 16.1 \times 10^5$  cells/ml  
4147 }

NOTE: RESPEND CELLS IN 2ml D-medium after  (i.e. WASHING)

PLATED: 0.2ml + 0.2ml = 0.4ml (i.e.  $\approx 6.44 \times 10^5$ )

Time: 9:50

24					12:50
44					13:50
64					15:50
84					17:50

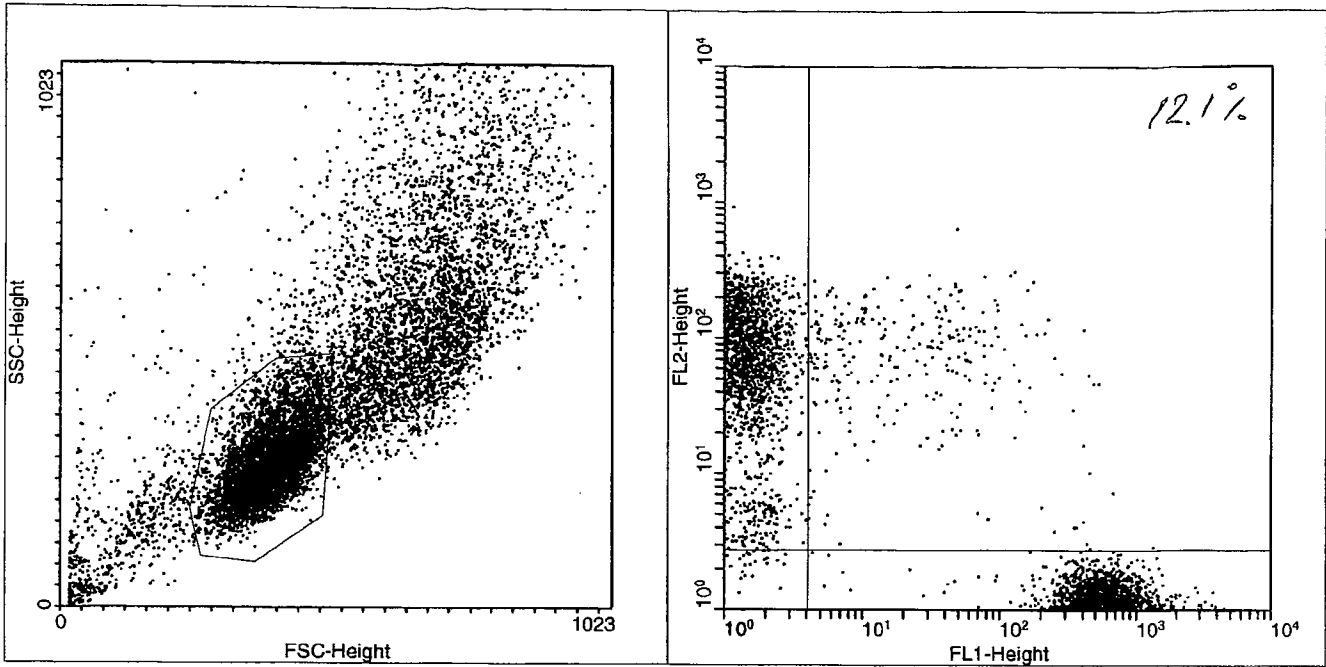
COUNTER COUNTS OF 2h CULTURE (AFTER FIXATION)  
1.5 ml - aliquots

CA / DiI ~~429~~ 275 }  
256 }  $272 \times 400 \approx 1.1 \times 10^5$  cells/ml  
284 }

CA\* / DiI 305 }  
295 }  $298 \times 400 \approx 1.2 \times 10^5$  cells/ml  
294 }

CA / DiI\* 374 }  
392 }  $384 \times 400 \approx 1.5 \times 10^5$  cells/ml  
386 }

CA\* / DiI\* 463 }  
465 }  $461 \times 400 \approx 1.8 \times 10^5$  cells/ml  
454 }

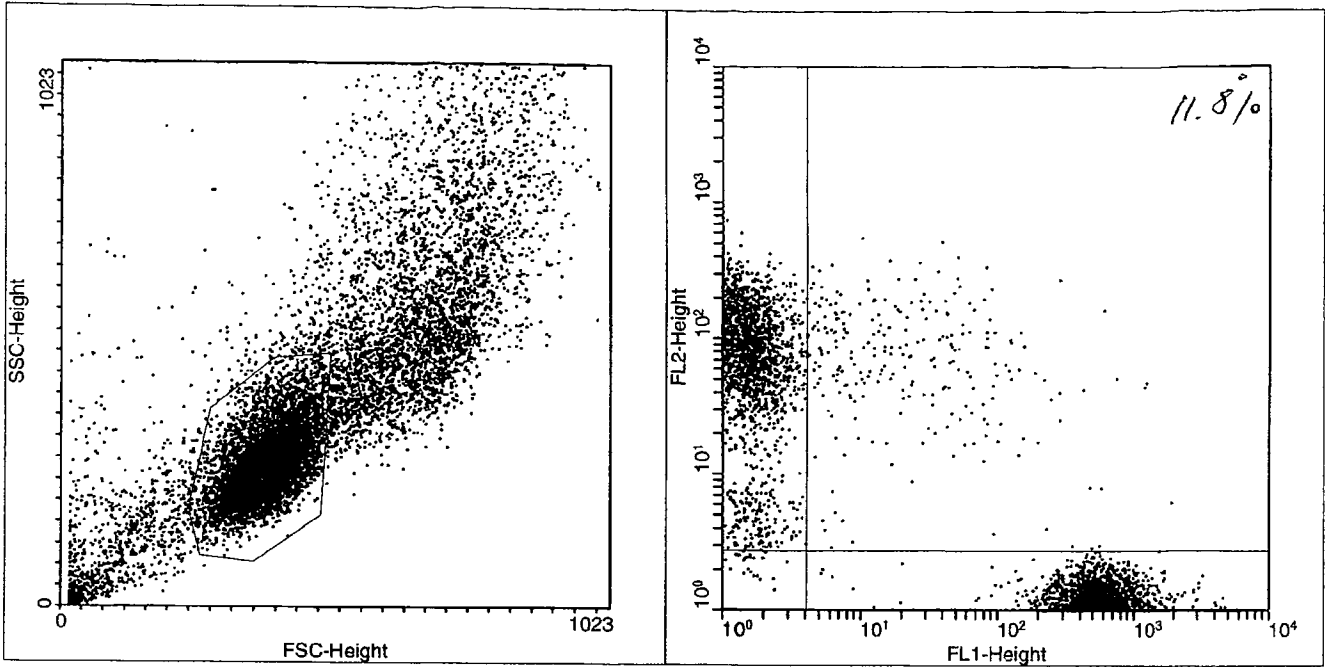


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:01:18 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041502 Sample: WB CA/DiI Ogy 2h  
 Date: 15-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 10145 50.72%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	71.6	3986	19.93	39.29
2 UR	22.1	68.1	550	2.75	5.42
3 LL	1.5	2.1	50	0.25	0.49
4 LR	606.4	1.1	5559	27.80	54.80

} 44.71 → 12.1%



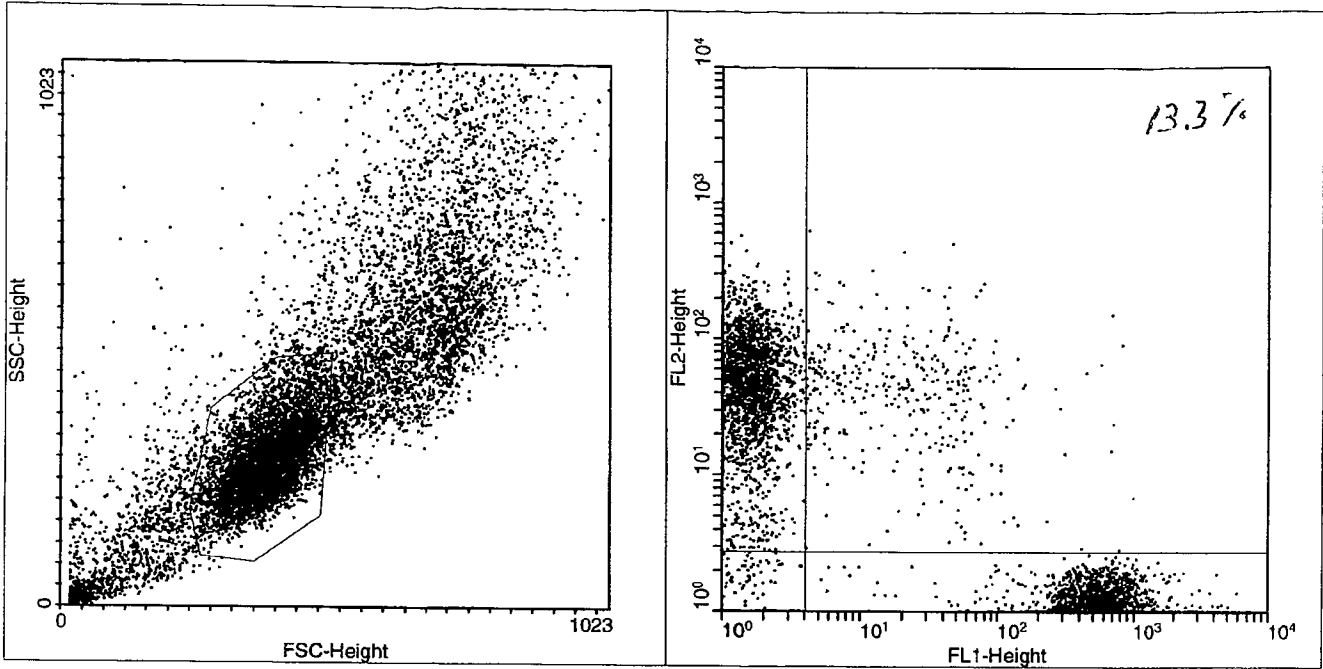
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:04:39 2002  
 Gates: R1

Project: Experiment:  
 File: 041502 Sample: WB CA\*/DiI 9Gy 2h  
 Date: 15-Apr-2 Parameters: /  
 Total Events 20000 Gated Events 10085 50.42%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	71.0	3816	19.08	37.84
2 UR	21.9	65.0	508	2.54	5.04
3 LL	1.7	2.1	51	0.26	0.51
4 LR	607.7	1.1	5710	28.55	56.62

42.88 → 11.8%



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:06:53 2002

Gates: R1

Project: Experiment:

File: 041502 Sample: WB CA/DiI\* 9Gy 2h

Date: 15-Apr-2 Parameters: 7

Total Events 20000 Gated Events 9730 48.65%

System: Log Parameter Means: Geometric

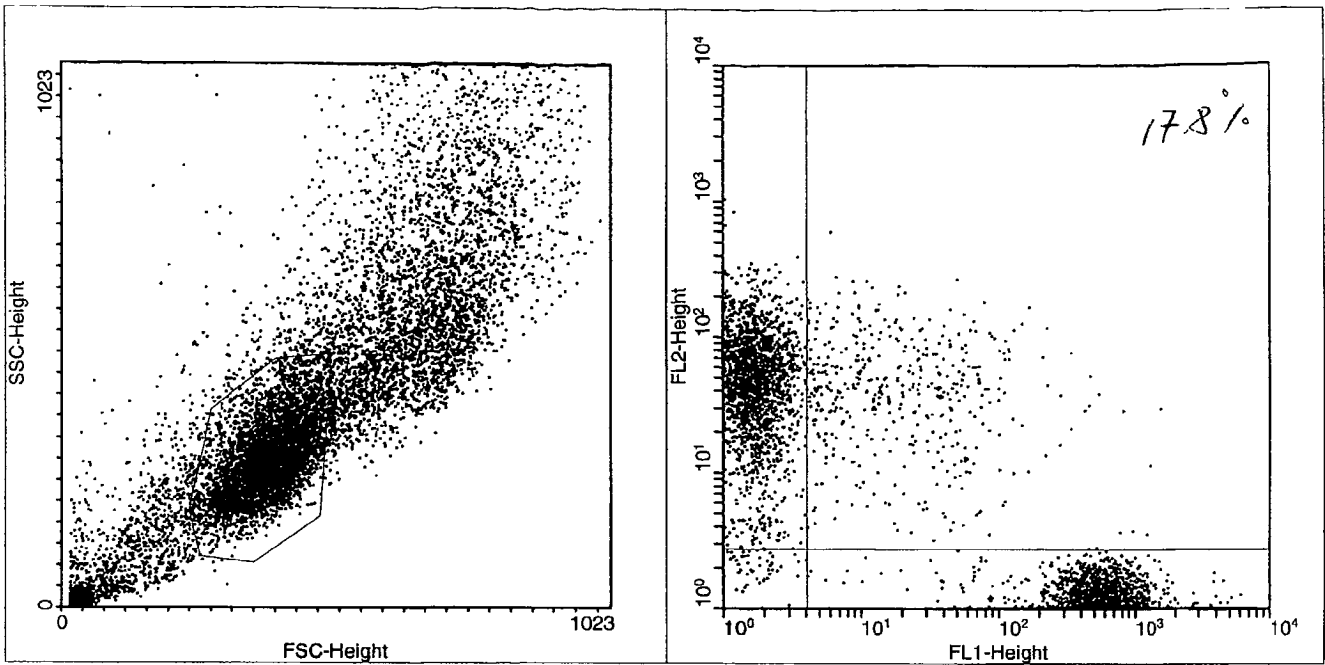
Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	45.1	4557	22.79	46.83
2 UR	21.0	38.4	698	3.49	7.17
3 LL	1.5	1.9	129	0.65	1.33
4 LR	608.1	1.1	4346	21.73	44.67

54.0 → 13.3



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:08:48 2002

Gates: R1

Project: Experiment:

File: 041502 Sample: WB CA\*/DiI\* 9Gy 2h

Date: 15-Apr-2 Parameters: 7

Total Events 20000 Gated Events 9255 46.28%

System: Log Parameter Means: Geometric

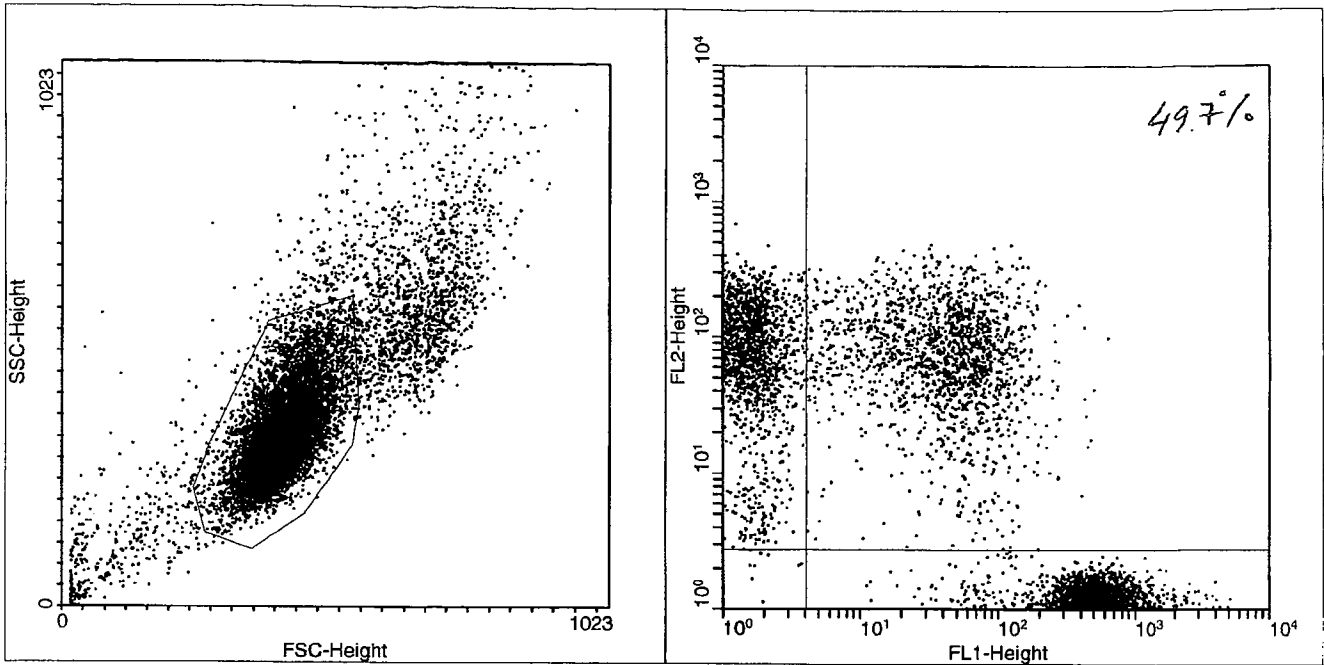
Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	45.0	3900	19.50	42.14
2 UR	20.2	38.6	845	4.23	9.13
3 LL	1.7	2.0	107	0.53	1.16
4 LR	591.4	1.2	4403	22.02	47.57

} 51.27 → 17.8



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:13:42 2002

Gates: R1

Project: Experiment:

File: 041502 Sample: WB CA/DiI 0Gy 4h

Date: 15-Apr-2 Parameters: 7

Total Events 20000 Gated Events 15678 78.39%

System: Log Parameter Means: Geometric

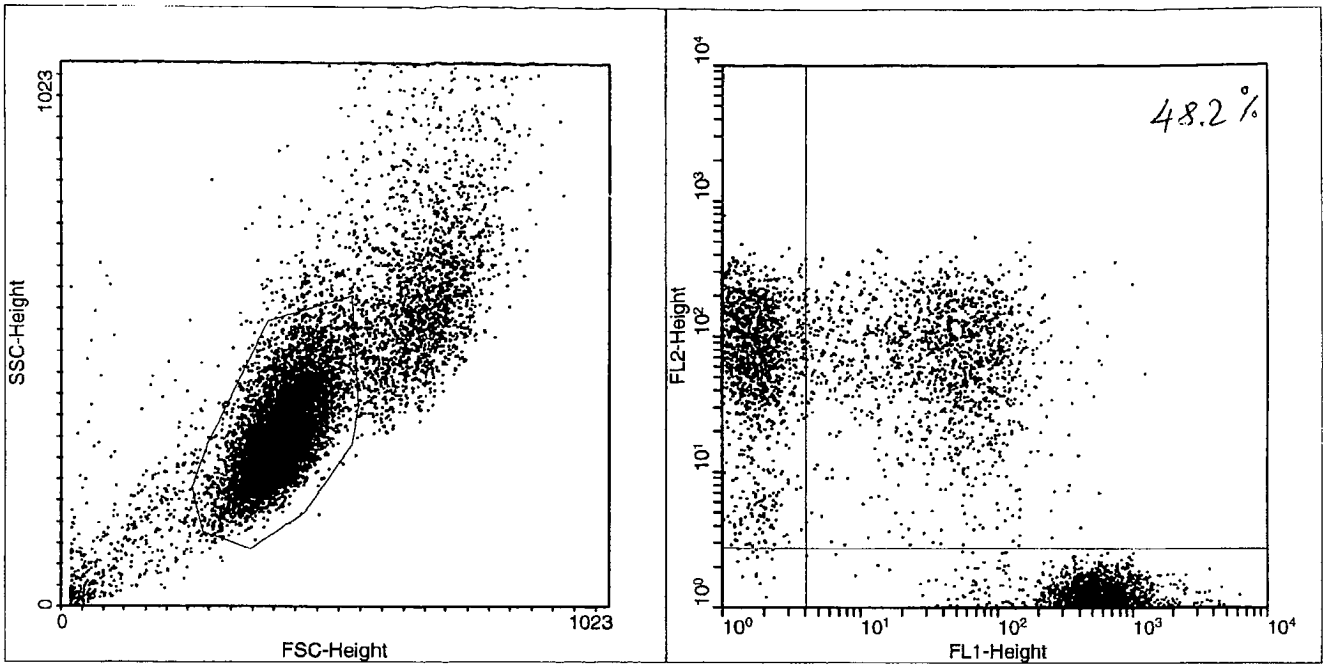
Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	77.1	3359	16.80	21.42
2 UR	38.0	68.3	3323	16.62	21.20
3 LL	1.7	1.9	24	0.12	0.15
4 LR	530.1	1.1	8972	44.86	57.23

42.62 → 49.7



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:15:20 2002

Gates: R1

Project: Experiment:

File: 041502 Sample: WB CA\*/DiI 9Gy 4h

Date: 15-Apr-2 Parameters: 7

Total Events 20000 Gated Events 14435 72.18%

System: Log Parameter Means: Geometric

Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

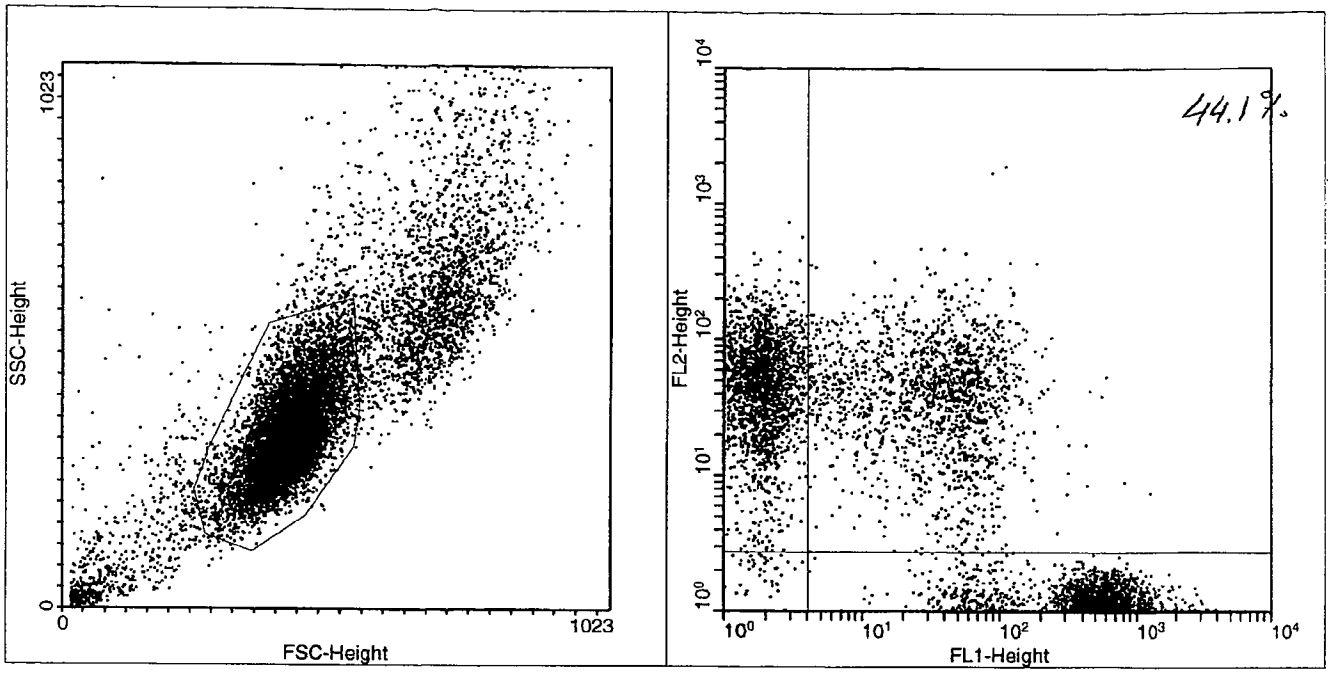
Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.7	71.8	3023	15.12	20.94
2 UR	40.2	66.2	2808	14.04	19.45
3 LL	1.8	2.1	27	0.14	0.19
4 LR	574.6	1.1	8577	42.89	59.42

} 40.39 → 48.2

↑

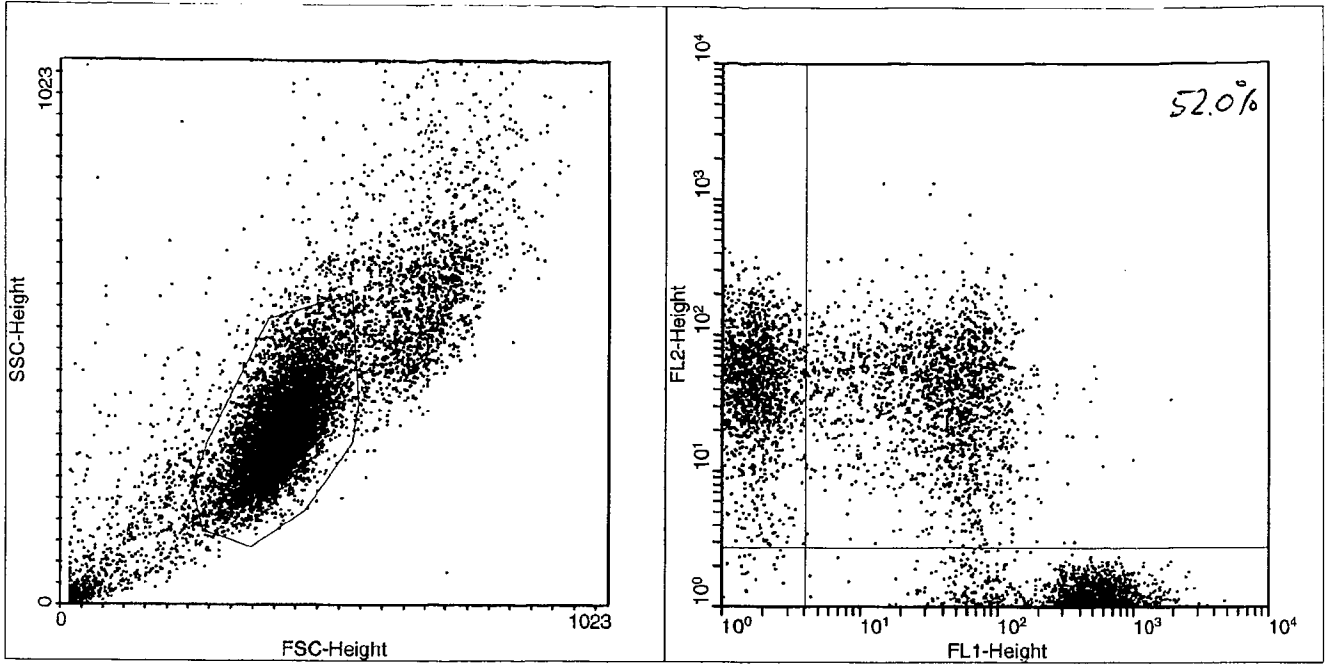




Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:15:57 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041502 Sample: WB CA/DiI\* 9Gy 4h  
 Date: 15-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13980 69.90%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 156,112  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.8	45.5	3887	19.44	27.80
2 UR	30.8	36.7	3062	15.31	21.90
3 LL	1.8	1.9	94	0.47	0.67
4 LR	479.0	1.1	6937	34.69	49.62

} 49.7 → 44.1  
 ↑



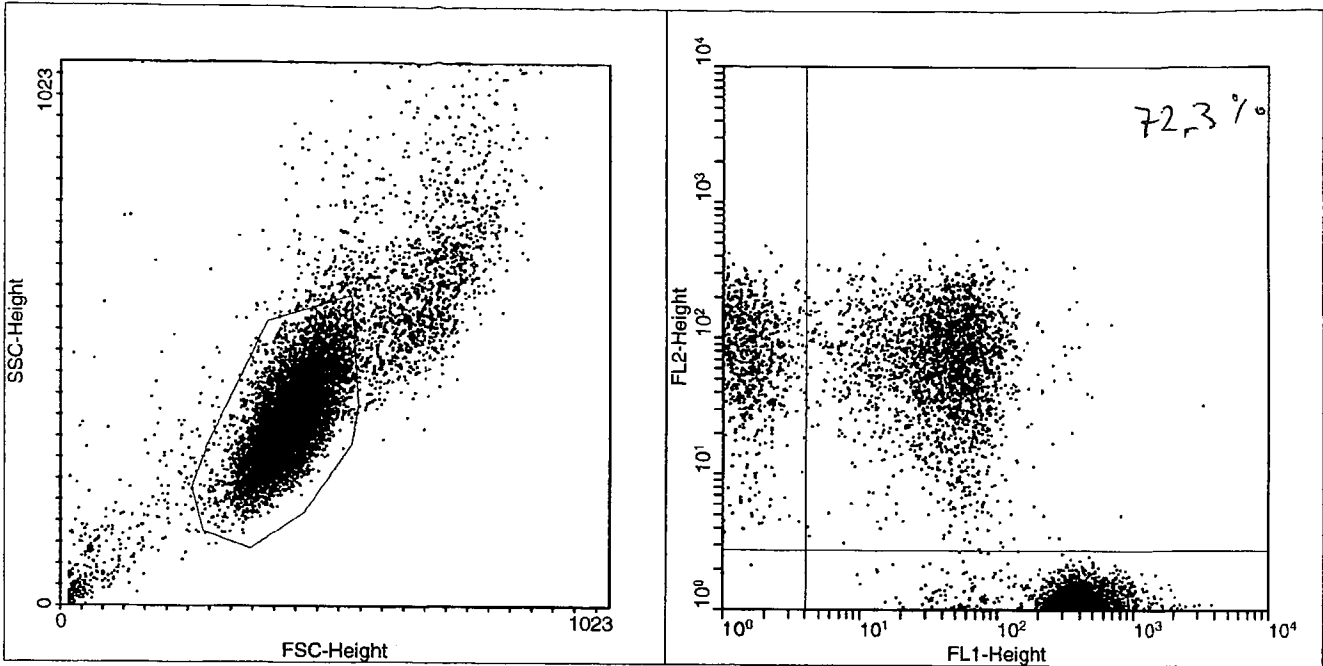
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:16:40 2002  
 Gates: R1

Project: Experiment:  
 File: 041502 Sample: (WB CA\*/DiI\* 9Gy 4h)  
 Date: 15-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14528 72.64%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.7	46.2	3335	16.68	22.96
2 UR	36.8	36.7	3609	18.05	24.84
3 LL	1.6	2.0	51	0.26	0.35
4 LR	482.7	1.1	7533	37.67	51.85

} 47.8 → 52.0  
↑



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:18:06 2002

Gates: R1

Project: Experiment:

File: 041502 Sample: WB CA/DiI 0Gy 6h

Date: 15-Apr-2 Parameters: 7

Total Events 20000 Gated Events 16316 81.58%

System: Log Parameter Means: Geometric

Quad Stats

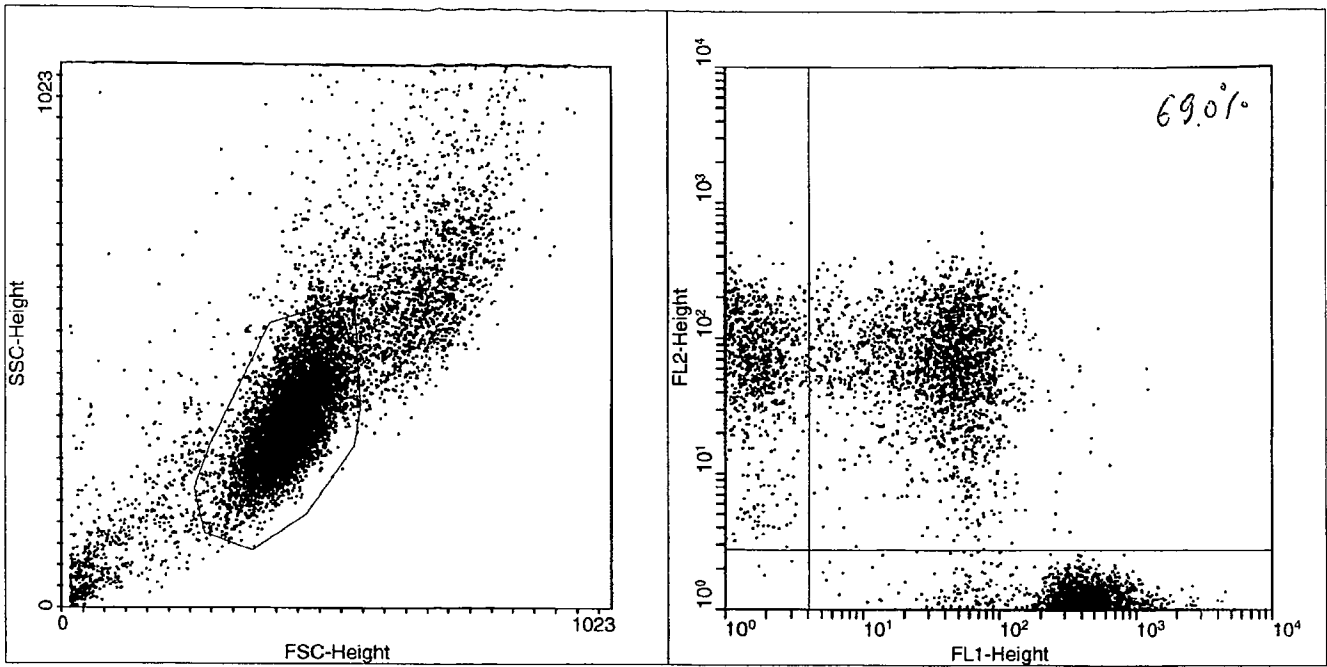
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	73.3	1882	9.41	11.53
2 UR	39.1	64.8	4907	24.54	30.07
3 LL	1.5	2.2	6	0.03	0.04
4 LR	386.4	1.1	9521	47.61	58.35

41.6 → 72.3

↑



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:18:36 2002

Gates: R1

Project: Experiment

File: 041502 Sample: WB CA\*/DII 9Gy 6h

Date: 15-Apr-2 Parameters: 7

Total Events 20000 Gated Events 14741 73.71%

System: Log Parameter Means: Geometric

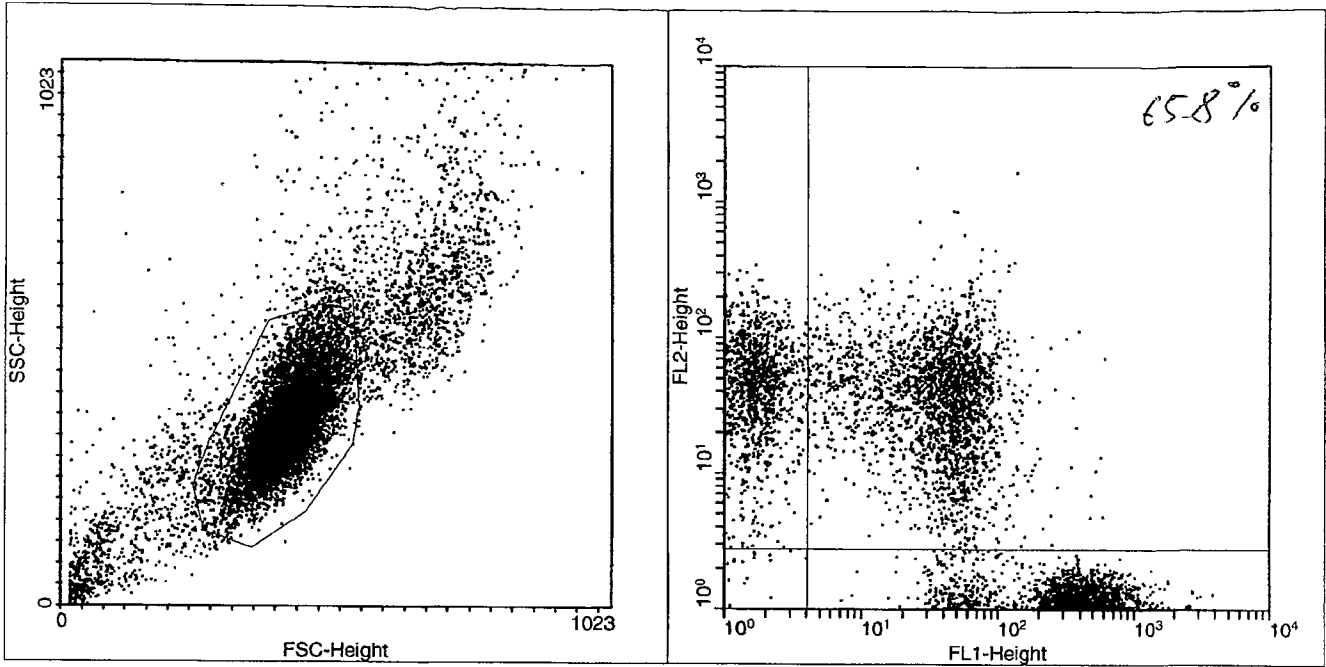
Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	73.7	1916	9.58	13.00
2 UR	37.3	63.4	4271	21.36	28.97
3 LL	1.8	2.0	7	0.04	0.05
4 LR	418.7	1.1	8547	42.74	57.98

41.97 → 69.0%  
 NO CHANGE



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:21:47 2002

Gates: R1

Project: Experiment:

File: 041502 Sample: WB CA/DiI\* 9Gy 6h

Date: 15-Apr-2 Parameters: 7

Total Events 20000 Gated Events 15462 77.31%

System: Log Parameter Means: Geometric

Quad Stats

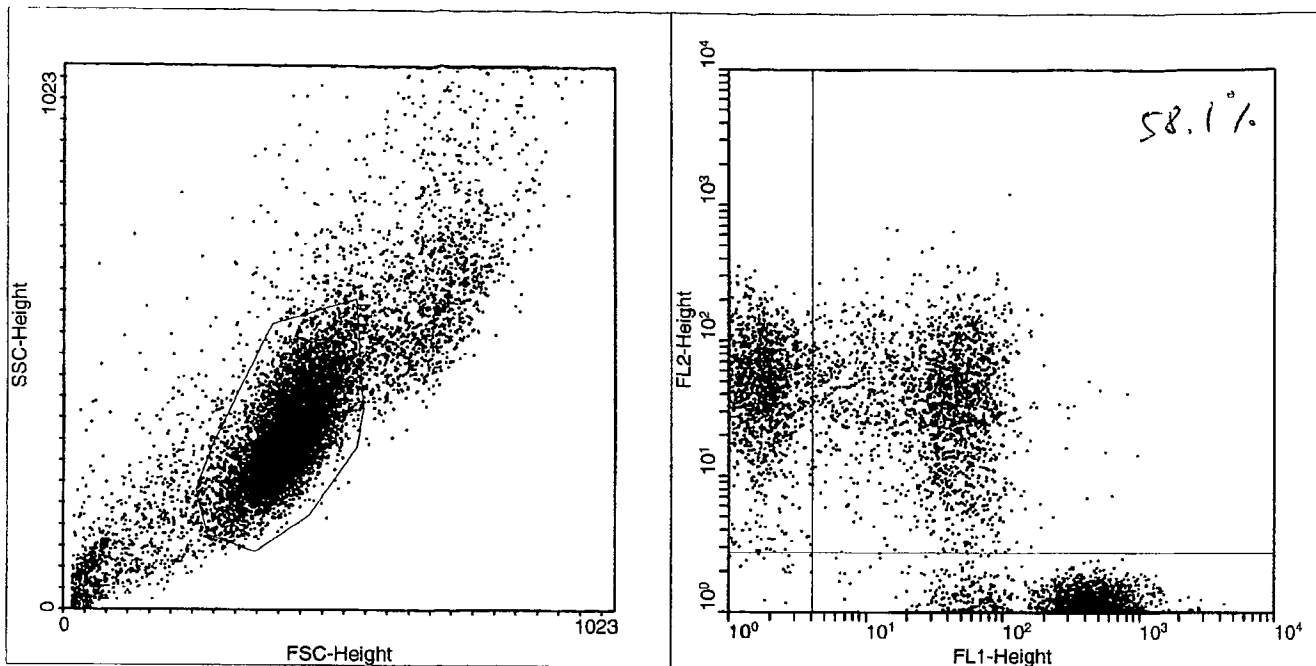
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	45.4	2445	12.23	15.81
2 UR	34.0	34.6	4695	23.48	30.36
3 LL	1.5	2.1	35	0.18	0.23
4 LR	356.2	1.1	8287	41.44	53.60

} 46.17 → 65.8

↑



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:22:51 2002

Gates: R1

Project: Experiment:

File: 041502 Sample: WB CA\*/DiI\* 9Gy 6h

Date: 15-Apr-2 Parameters: 7

Total Events 20000 Gated Events 14827 74.14%

System: Log Parameter Means: Geometric

Quad Stats

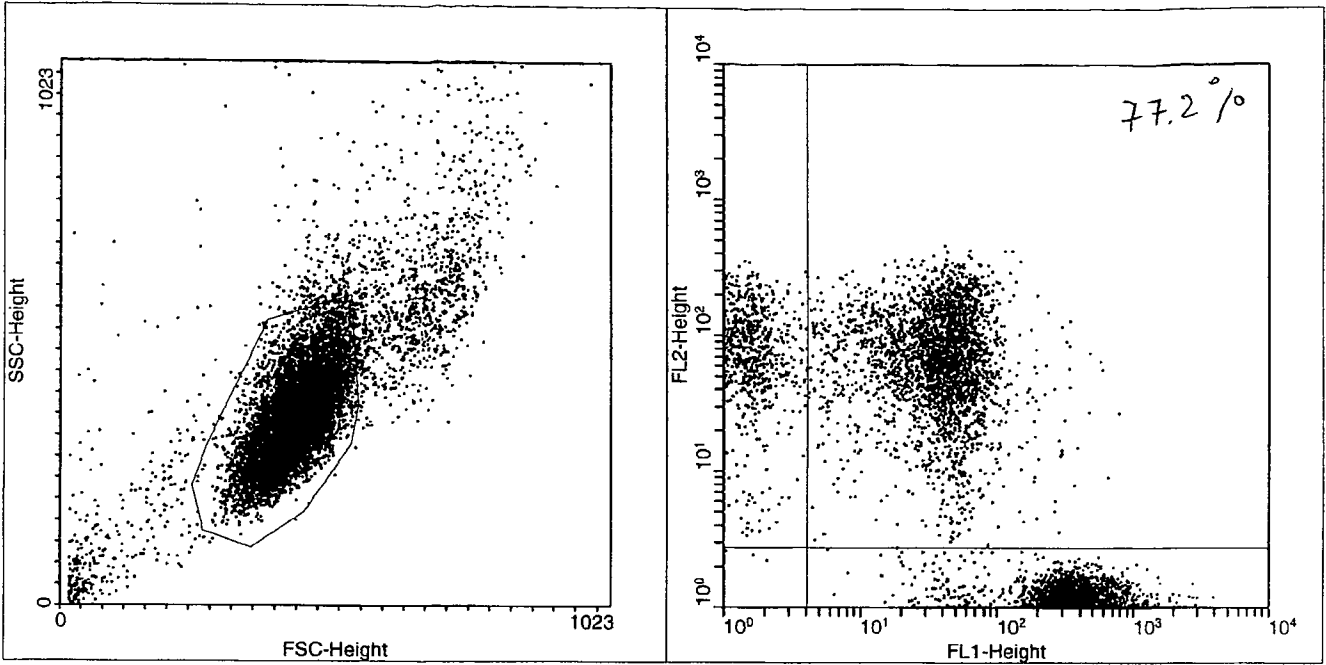
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.8	45.8	2929	14.65	19.75
2 UR	32.6	36.2	4065	20.33	27.42
3 LL	1.8	2.0	39	0.20	0.26
4 LR	392.7	1.1	7794	38.97	52.57

} ~~47.17~~ → 58.1

↑



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:23:37 2002

Gates: R1

Project: Experiment:

File: 041502 Sample: WB CA/DiI 0Gy 8h

Date: 15-Apr-2 Parameters: 7

Total Events 20000 Gated Events 16846 84.23%

System: Log Parameter Means: Geometric

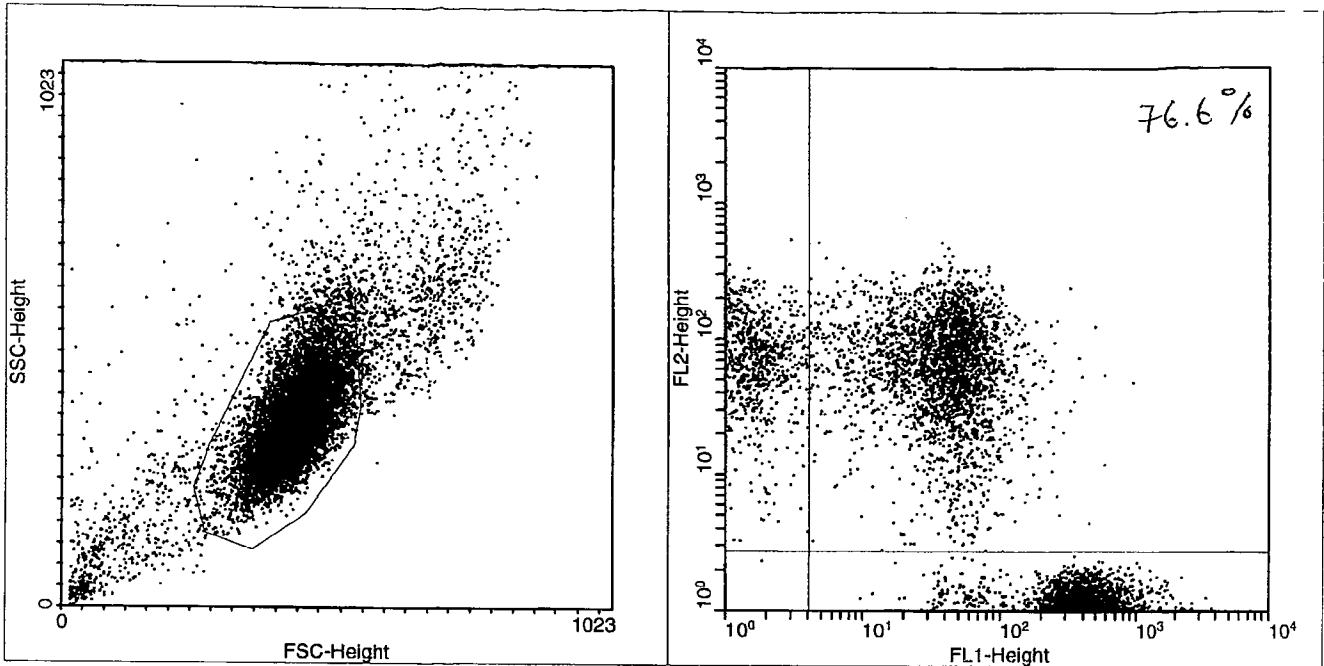
Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	73.3	1625	8.13	9.65
2 UR	36.2	64.0	5513	27.57	32.73
3 LL	1.4	2.4	5	0.03	0.03
4 LR	345.1	1.1	9703	48.52	57.60

} 42.38 → 77.2%  
 NO CHANGE



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:24:44 2002  
 Gates: R1

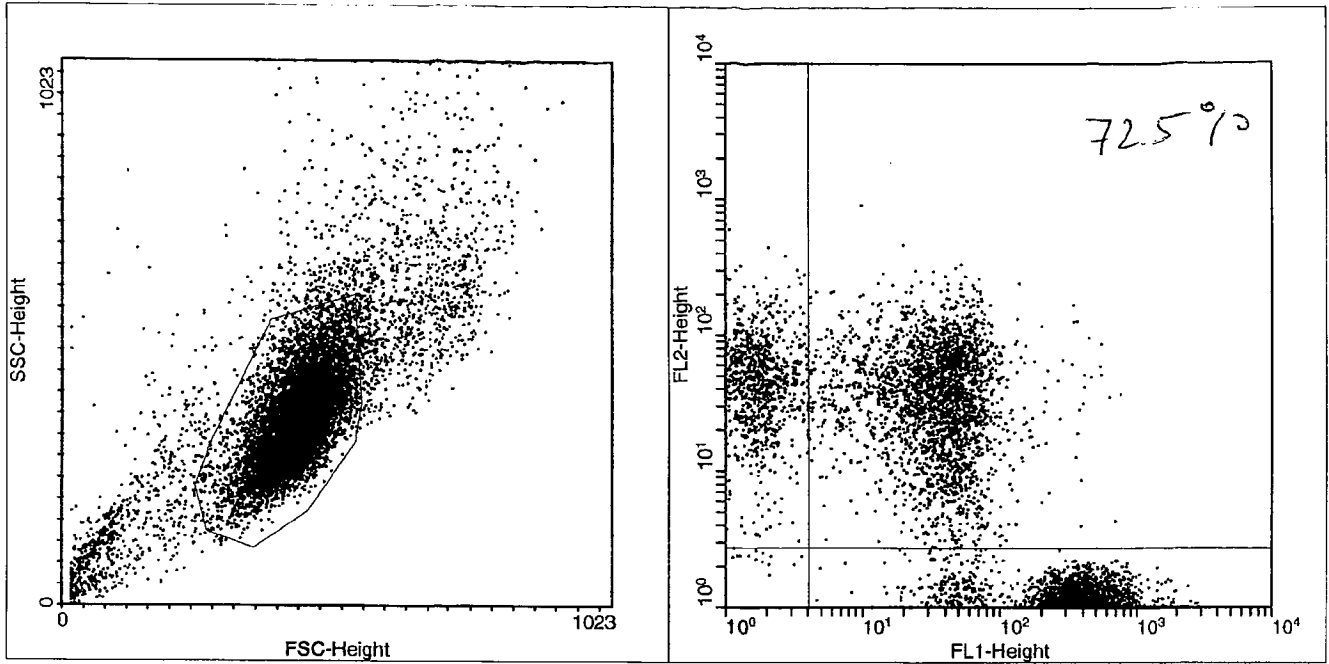
Project: Experiment;  
 File: 041502 Sample: (WB CA\*/DiI 9Gy 8h)  
 Date: 15-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16592 82.96%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	74.3	1631	8.15	9.83
2 UR	37.8	61.3	5350	26.75	32.24
3 LL	1.6	2.1	4	0.02	0.02
4 LR	401.9	1.1	9607	48.04	57.90

42.07 → 76.6 %  
 NO CHANGE





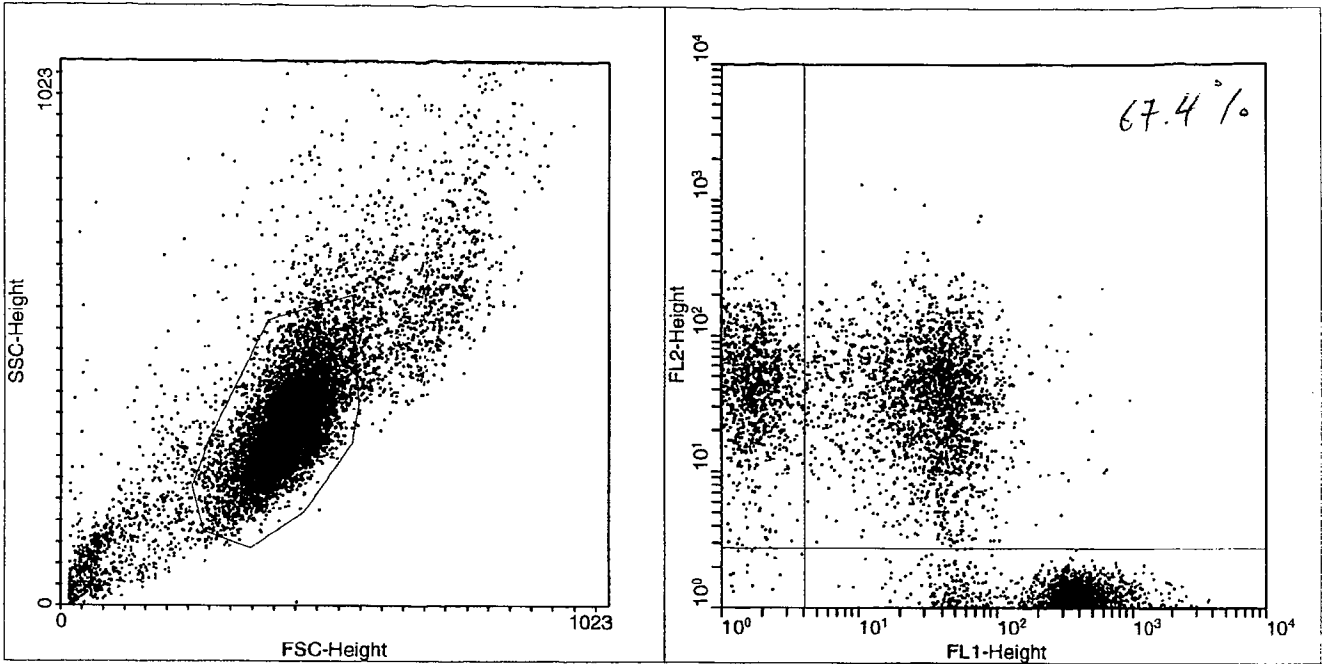
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:25:13 2002  
 Gates: R1

Project: Experiment:  
 File: 041502 Sample: WB CA/DiI\* 9Gy 8h  
 Date: 15-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16120 80.60%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.7	46.0	1936	9.68	12.01
2 UR	33.4	34.7	5101	25.51	31.64
3 LL	1.9	2.1	22	0.11	0.14
4 LR	347.7	1.1	9061	45.31	56.21

} 43.65 → 72.5  
 ↑



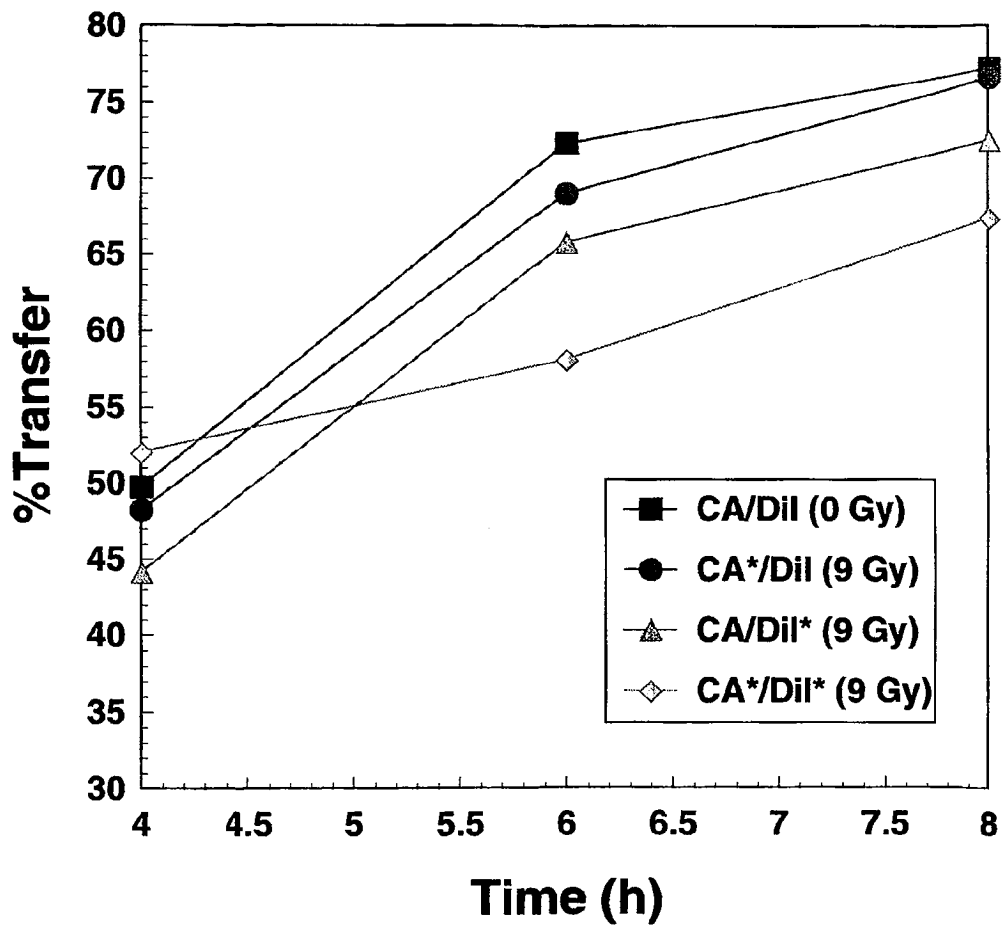
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 15 11:25:46 2002  
 Gates: R1

Project: Experiment:  
 File: 041502 Sample: WB CA\*/Dil\* 9Gy 8p  
 Date: 15-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 15455 77.28%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 156,112

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.7	43.7	2268	11.34	14.67
2 UR	32.3	34.6	4683	23.42	30.30
3 LL	1.6	1.9	26	0.13	0.17
4 LR	361.5	1.1	8478	42.39	54.86

44.97 → 67.4



Label	A	B	C	D	E	F
	Time (h)	1/DiI (0 G*/DiI	(9 G*/DiI	(9 G*/DiI*	(9 G*/DiI*	(9 G*/DiI*
1	0	0	0	0	0	0
2	2	12.1	11.8	13.3	17.8	
3	4	49.7	48.2	44.1	52	
4	6	72.3	69	65.8	58.1	
5	8	77.2	76.6	72.5	67.4	
6						

---

ATTN.

APRIL 16, 2002

CELL-TO-CELL COMMUNICATION (50/50 - STAINING)  
IRRADIATION (9 Gy) OF 50% POPULATION  
(REPLICATE EXPERIMENT)

WBS (31p)

IRRADIATION TIME:

COULTER COUNTS: (1ml Trypsin + 2ml D-medium)

9:30

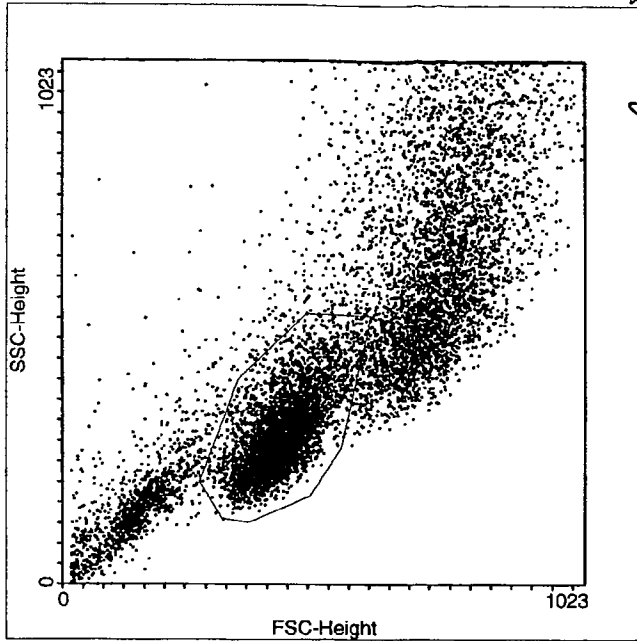
2801 }  
2722 } 2770 x 400  $\approx 11.1 \times 10^5$  cells/ml  
2787 }

0.4-ml aliquot (for plating) -  $\approx 4.4 \times 10^5$  cells

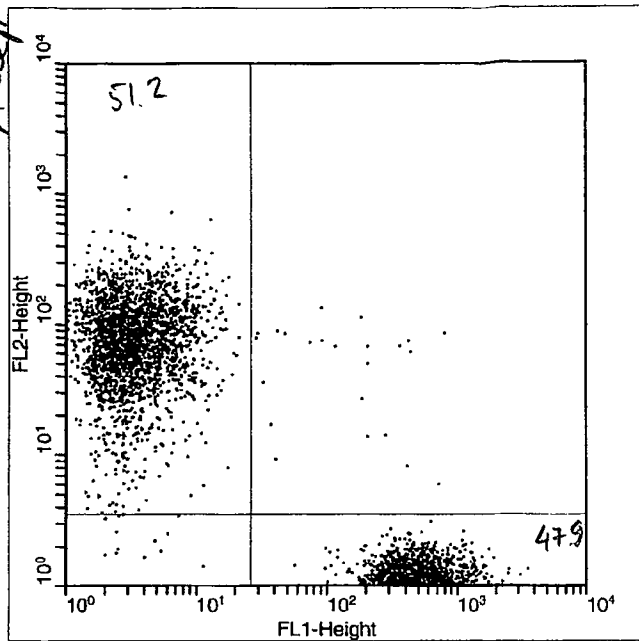
PLATING TIME: 10:30

HARVESTING: 14:30 (4h)  
16:30 (6h)  
18:30 (8h)

Note! CALCEIN-AM WAS ADDED AT  $\approx 5 \mu\text{M}$   
(20  $\mu\text{L}$   $\rightarrow$   $\approx 40 \text{ mL}$  D-medium)



Acceptor (A)

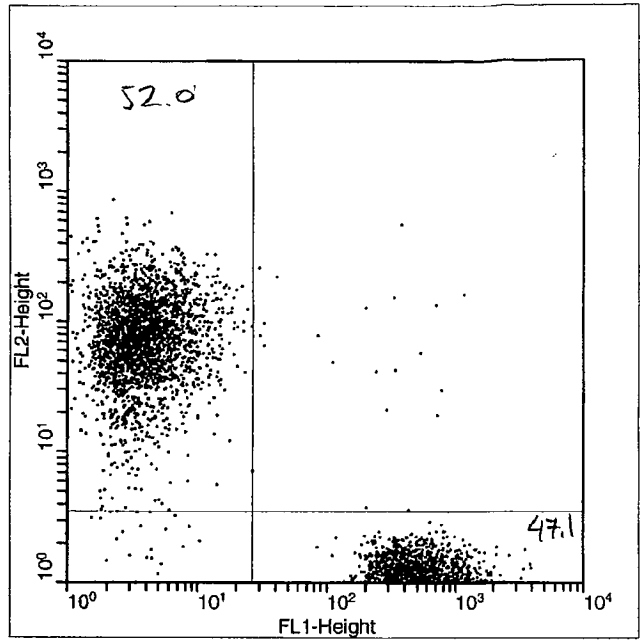
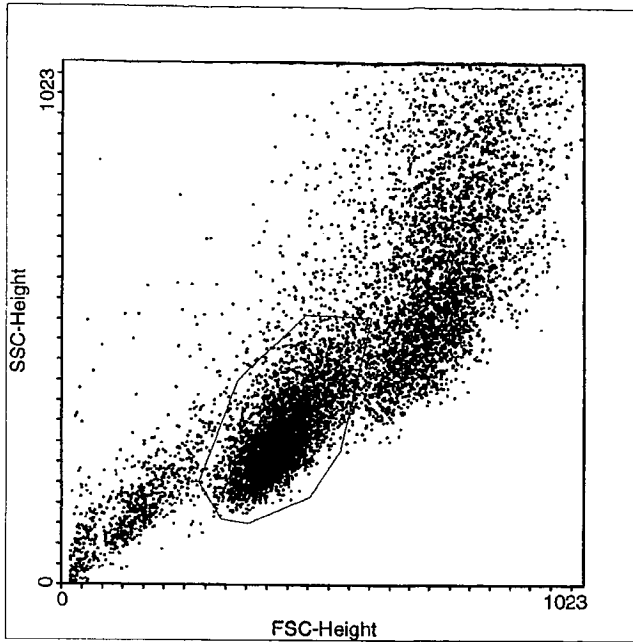


Donor (D)

Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 10:49:47 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: WB CA/DiI 0Gy 0h  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 8186 40.93%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x, y: 364,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.2	72.0	4192	20.96	51.21
2 UR	157.0	49.5	34	0.17	0.42
3 LL	3.4	2.2	40	0.20	0.49
4 LR	526.5	1.1	3920	19.60	47.89

$$\frac{\text{Acceptor (A)}}{\text{Donor (D)}} = 1.06$$

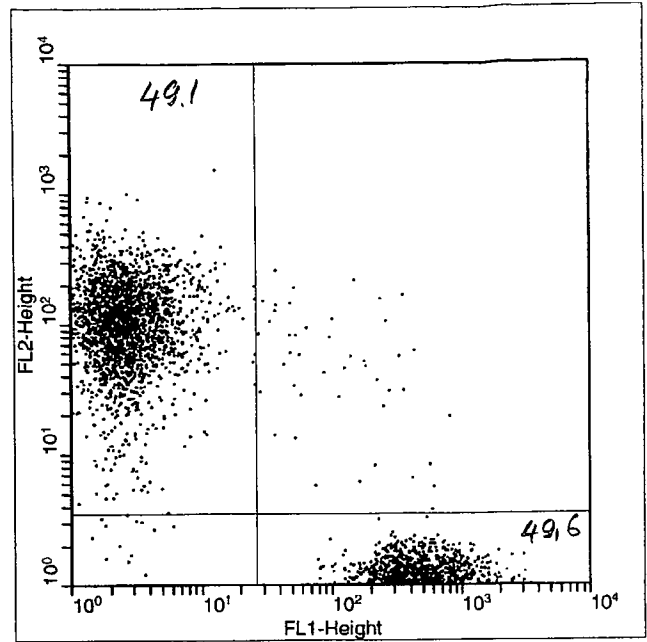
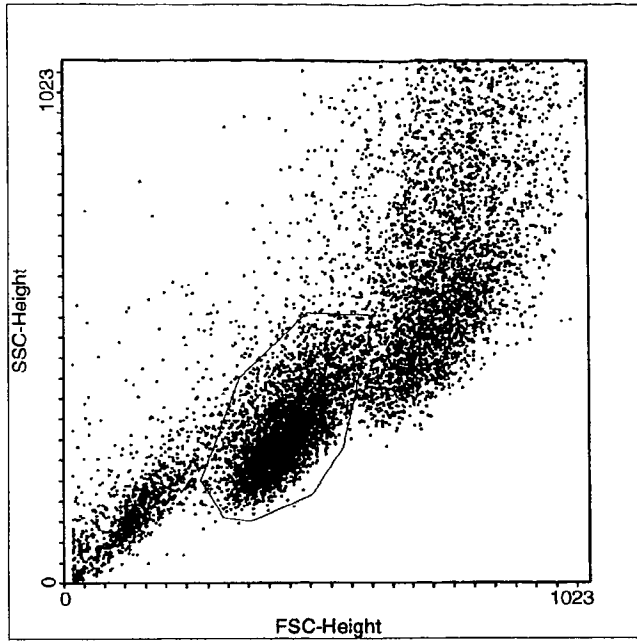


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 10:52:18 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: WB CA\*/DiI 9Gy 0h  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 8332 41.66%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 364,140  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.8	71.2	4332	21.66	51.99
2 UR	165.6	54.0	38	0.19	0.46
3 LL	4.2	2.1	41	0.21	0.49
4 LR	537.5	1.1	3921	19.61	47.06

$$A/D = 1.10$$

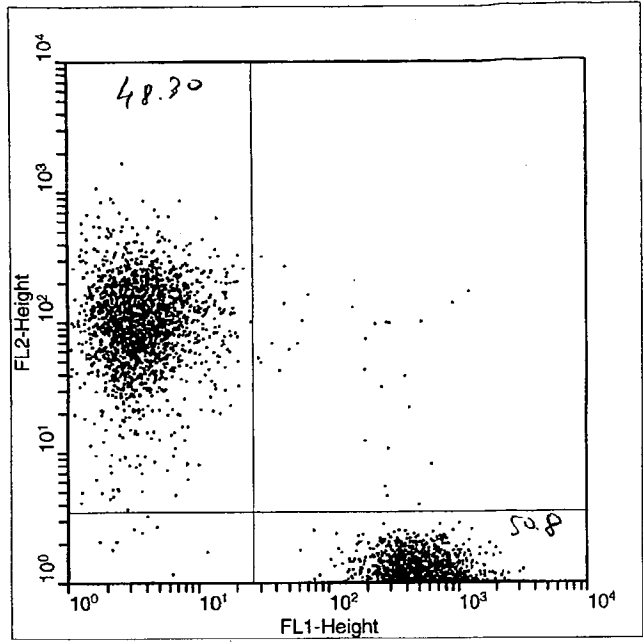
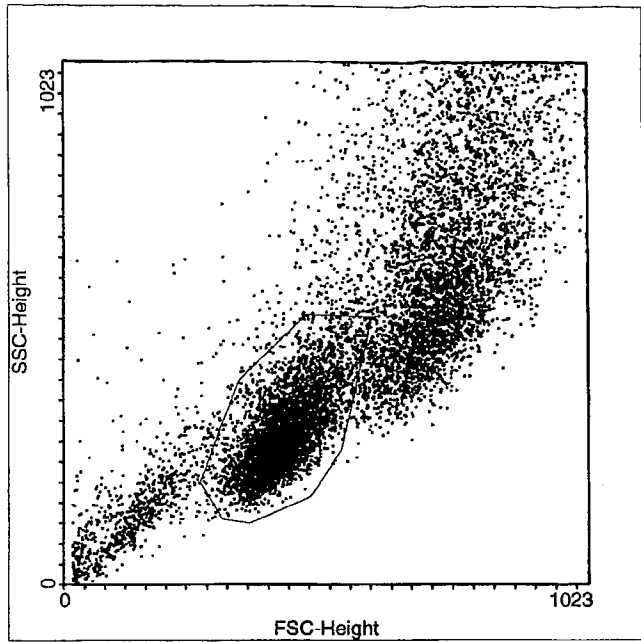




Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 10:53:08 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: WB CA/DiI\* 9Gy 0h  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 8293 41.47%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 364,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.7	99.9	4072	20.36	49.10
2 UR	122.0	46.8	75	0.38	0.90
3 LL	2.8	2.4	29	0.15	0.35
4 LR	480.1	1.1	4117	20.59	49.64

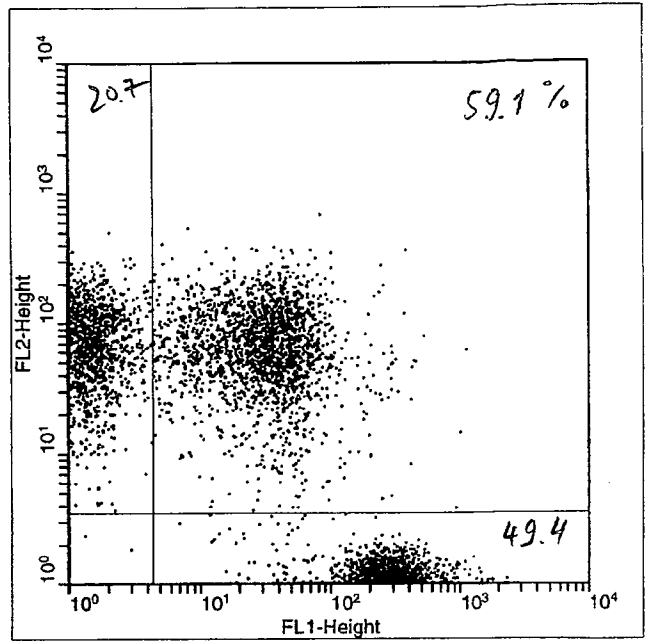
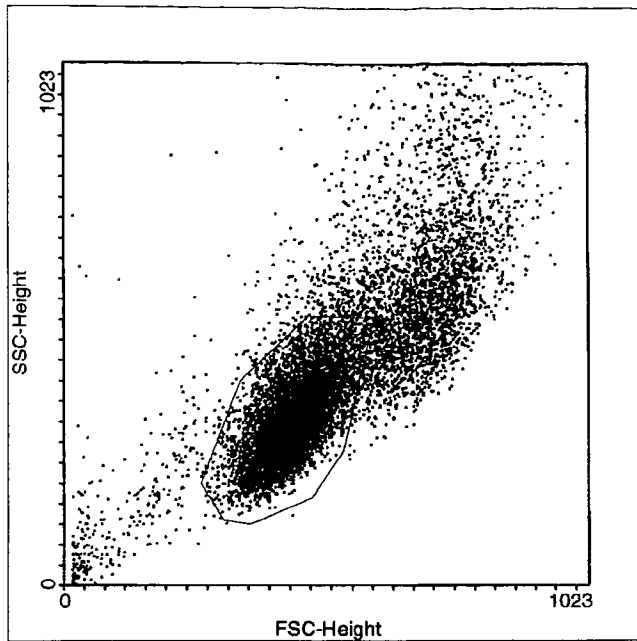
$$HD = 0.99$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 10:53:40 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: WB CA\*/DiI\* 9Gy 0h  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 8038 40.19%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 364,140  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.6	98.8	3882	19.41	48.30
2 UR	200.7	47.1	51	0.26	0.63
3 LL	3.9	2.1	22	0.11	0.27
4 LR	507.9	1.1	4083	20.42	50.80

$$A/D = 0.95$$

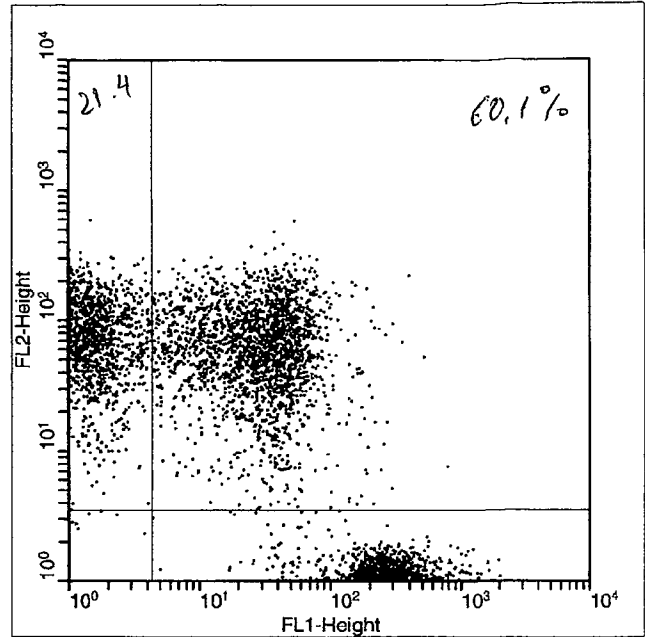
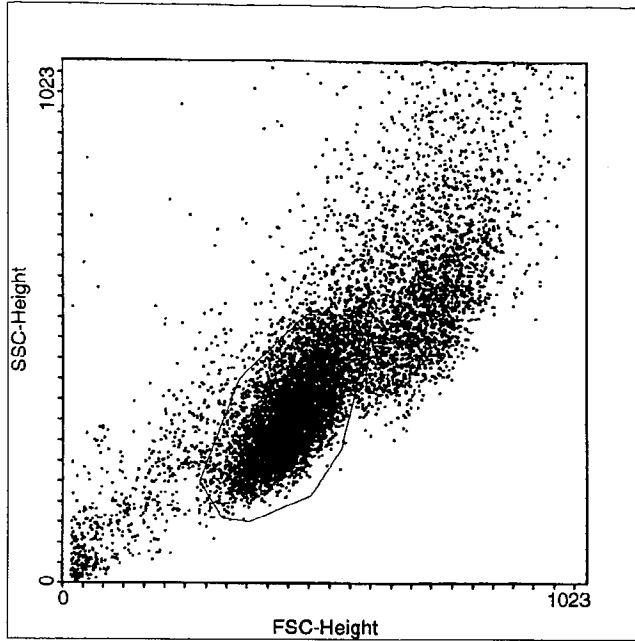


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 10:58:28 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: WB CA/DiI 0Gy 4h  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13169 65.85%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	71.9	2724	13.62	20.68
2 UR	29.2	61.1	3932	19.66	29.86
3 LL	1.4	2.3	5	0.03	0.04
4 LR	271.6	1.1	6508	32.54	49.42

50.54 → 59.1

A/D ~~2/A~~ = 1.02

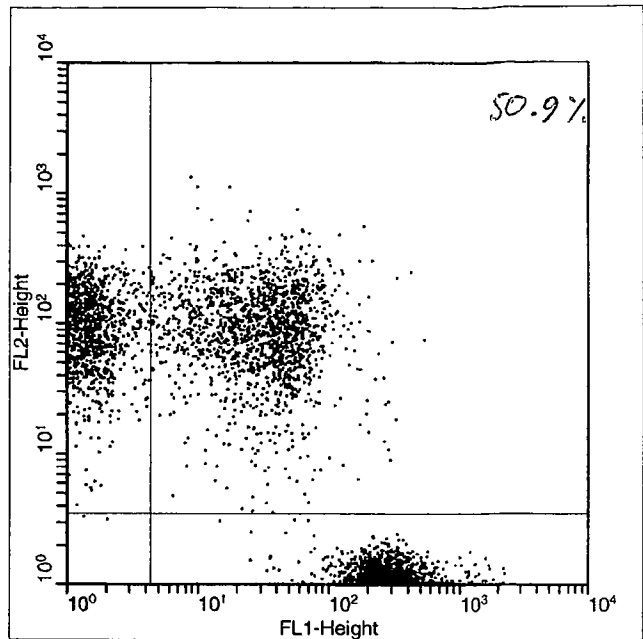
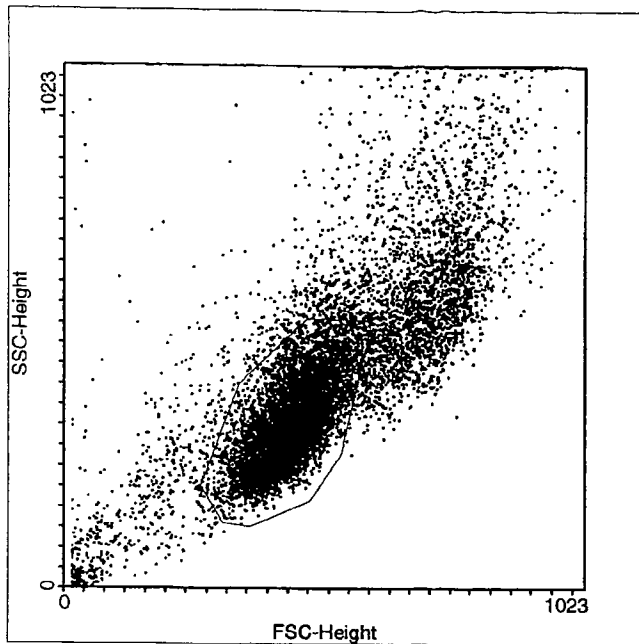


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 11:00:28 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: WB CA\*/DiI 9Gy 4h  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 12728 63.64%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	71.7	2728	13.64	21.43%
2 UR	27.5	59.5	4105	20.53	32.25%
3 LL	1.6	2.9	10	0.05	0.08%
4 LR	276.8	1.1	5885	29.43	46.24%

53.68 → 60.1

A/D  $\sigma/\mu = 1.16$



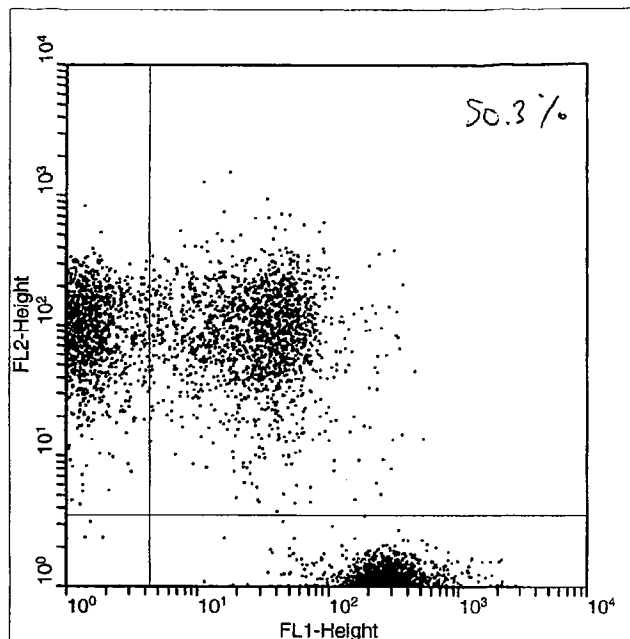
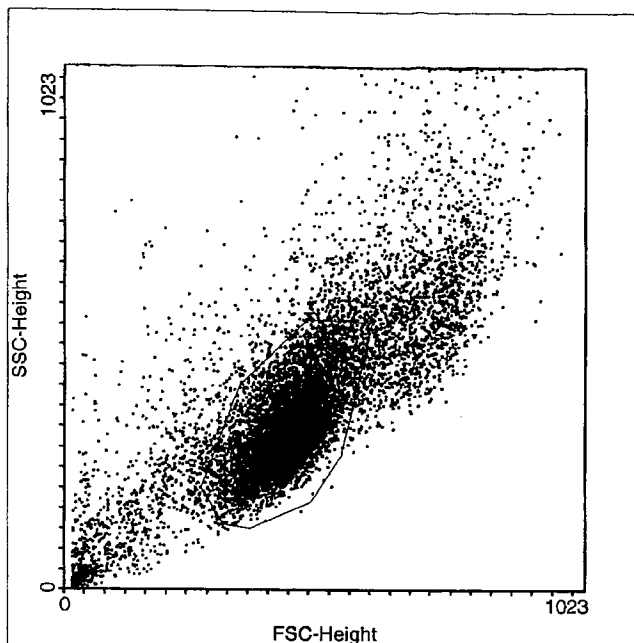
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 11:05:04 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: WB CA/DII\* 9Gy 4h  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 12950 64.75%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	99.9	2948	14.74	22.76
2 UR	29.9	85.9	3061	15.31	23.64
3 LL	1.4	3.0	3	0.02	0.02
4 LR	274.2	1.1	6938	34.69	53.58

} 46.4 → 50.9

$$A/D \quad D/A = \cancel{0.95} 0.86$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 11:06:27 2002

Gates: R1

Project: Experiment:

File: 041702 Sample: WB CA\*/DiI\* 9Gy 4h

Date: 17-Apr-2 Parameters: 7

Total Events 20000 Gated Events 13469 67.35%

System: Log Parameter Means: Geometric

Quad Stats

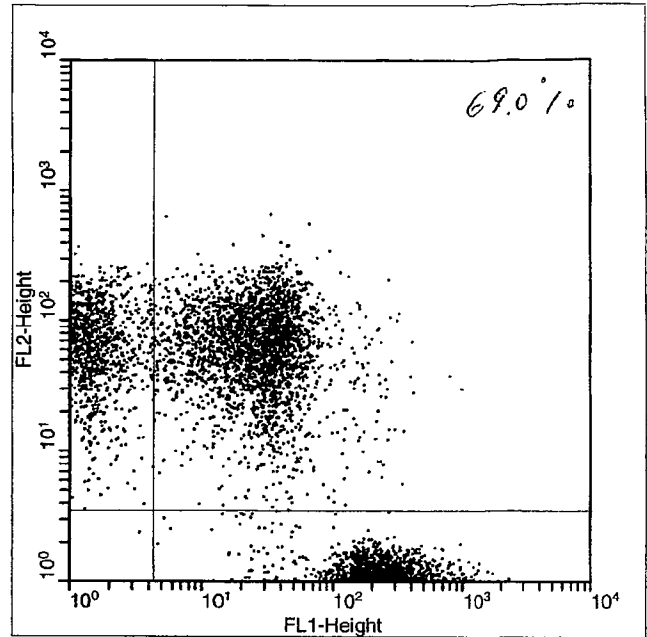
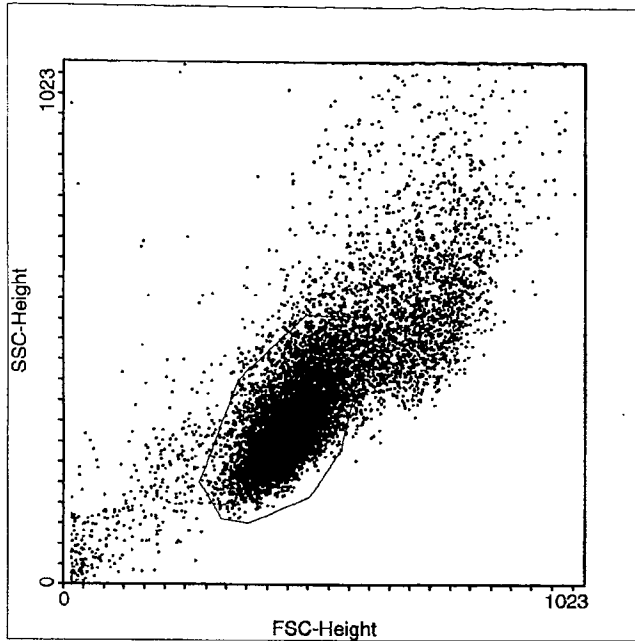
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	101.2	3244	16.22	24.08
2 UR	28.5	81.5	3286	16.43	24.40
3 LL	1.5	2.2	6	0.03	0.04
4 LR	284.9	1.1	6933	34.67	51.47

48.48 → 50.3

A/D ~~DA~~ = 0.94



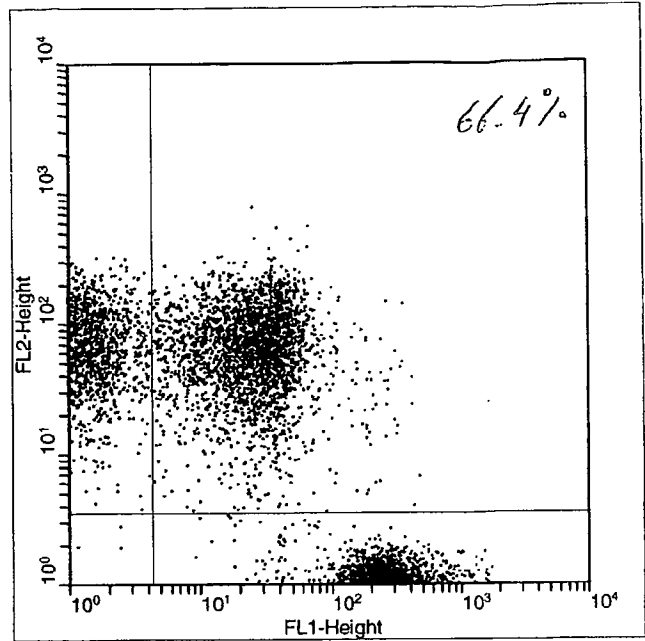
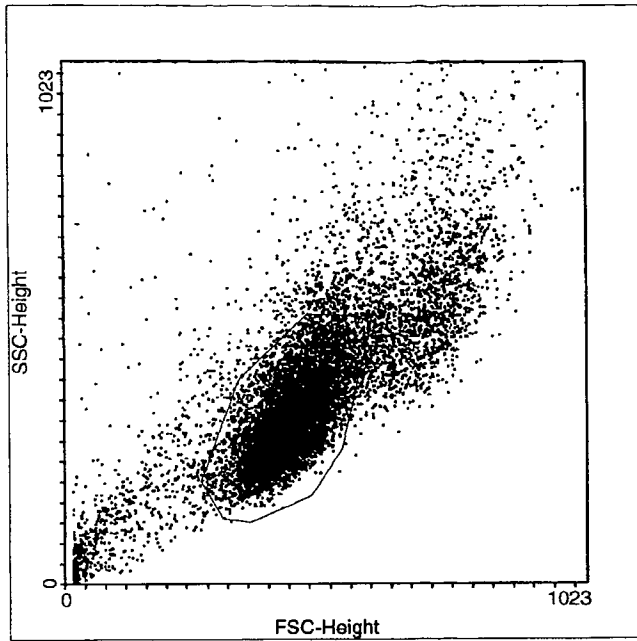
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 11:08:49 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: WB CA/DiI 0Gy 6h  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14550 72.75%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	66.8	2309	11.55	15.87
2 UR	26.2	58.9	5151	25.76	35.40
3 LL	2.4	2.3	3	0.02	0.02
4 LR	239.4	1.1	7087	35.44	48.71

} 51.27 → 69.0

A/D ~~DA~~ = 1.05



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 11:11:26 2002

Gates: R1

Project: Experiment:

File: 041702 Sample: WB CA\*/DiI 9Gy 6h

Date: 17-Apr-2 Parameters: 7

Total Events 20000 Gated Events 13902 69.51%

System: Log Parameter Means: Geometric

Quad Stats

FL1-Height (Log) vs FL2-Height (Log)

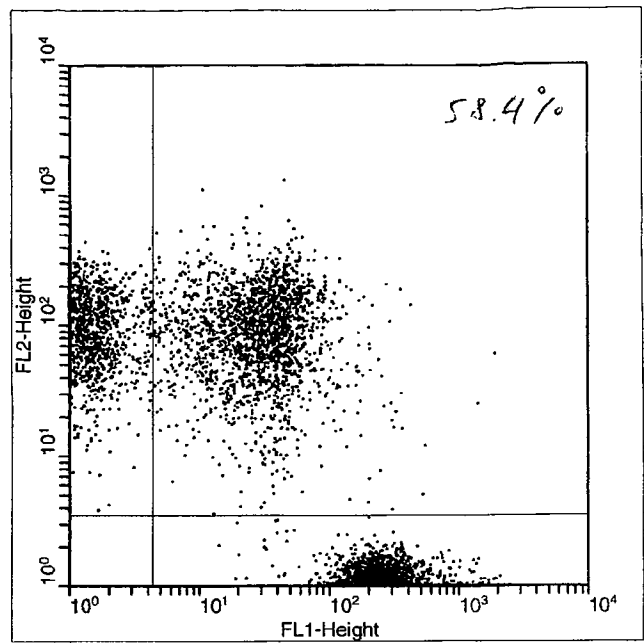
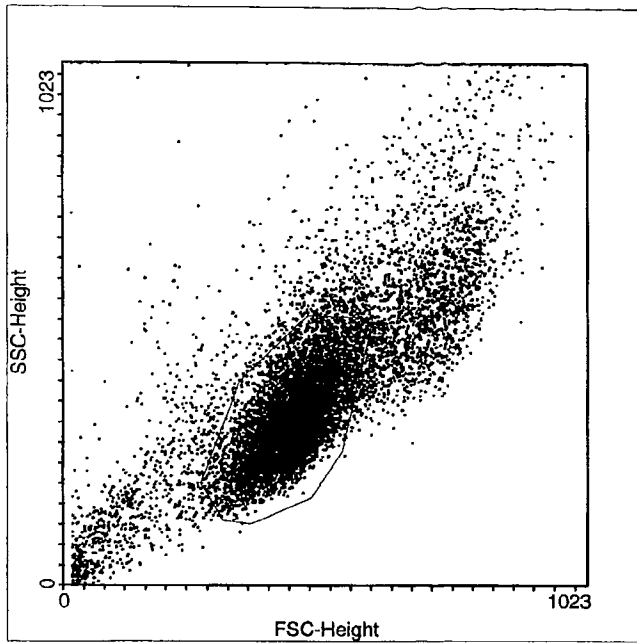
Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	67.4	2562	12.81	18.43
2 UR	24.8	58.7	5058	25.29	36.38
3 LL	1.9	2.4	7	0.04	0.05
4 LR	250.3	1.1	6275	31.38	45.14

} 54.81 → 66.4

A/D ~~DA~~ = 1.21



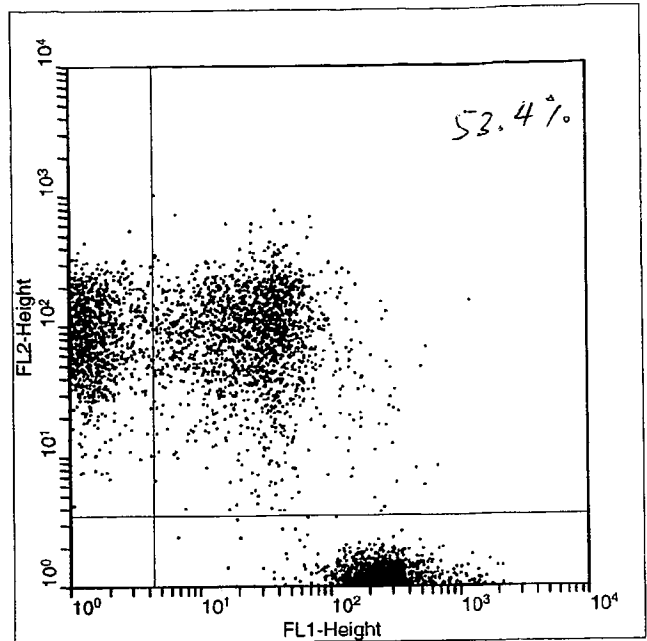
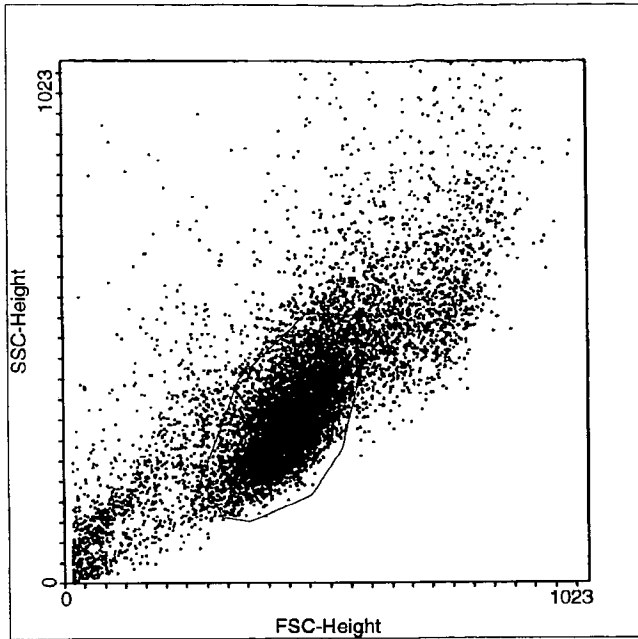


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 11:11:56 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: (WB CA/DII\* 9Gy 6h)  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13891 69.46%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	96.9	2596	12.98	18.69
2 UR	27.8	82.3	3646	18.23	26.25
3 LL	1.2	2.8	2	0.01	0.01
4 LR	255.4	1.1	7647	38.24	55.05

44.94 → 58.4

A/D ~~D/A~~ = 0.82

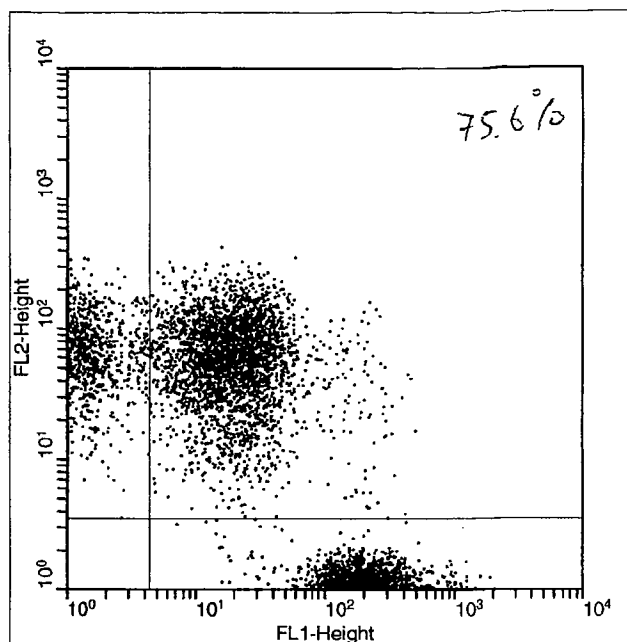
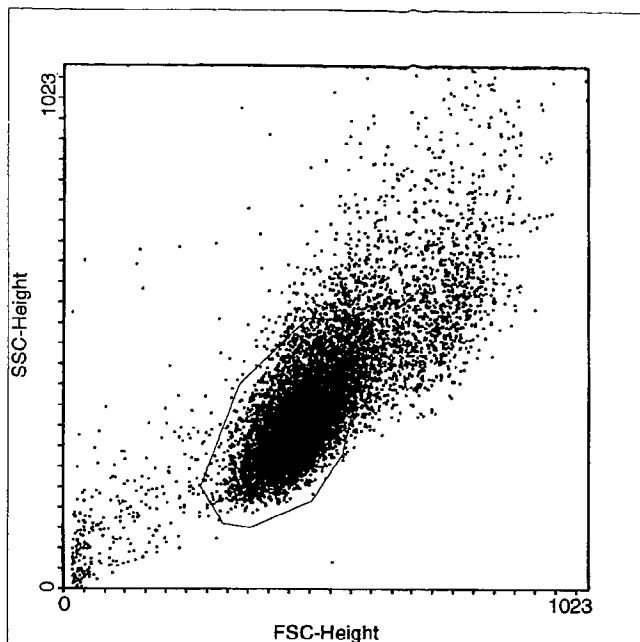


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 11:12:21 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: (WB CA\*/DiI\* 9Gy 6h)  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13561 67.81%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	93.7	3085	15.43	22.75
2 UR	25.3	81.2	3539	17.70	26.10
3 LL	0.0	0.0	0	0.00	0.00
4 LR	254.7	1.1	6937	34.69	51.15

48.85 → 53.4

A/D BTA = 0.96



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 11:12:45 2002

Gates: R1

Project: Experiment:

File: 041702 Sample: WB CA/DiI Ogy 8h

Date: 17-Apr-2 Parameters: /

Total Events 20000 Gated Events 15584 77.92%

System: Log Parameter Means: Geometric

Quad Stats

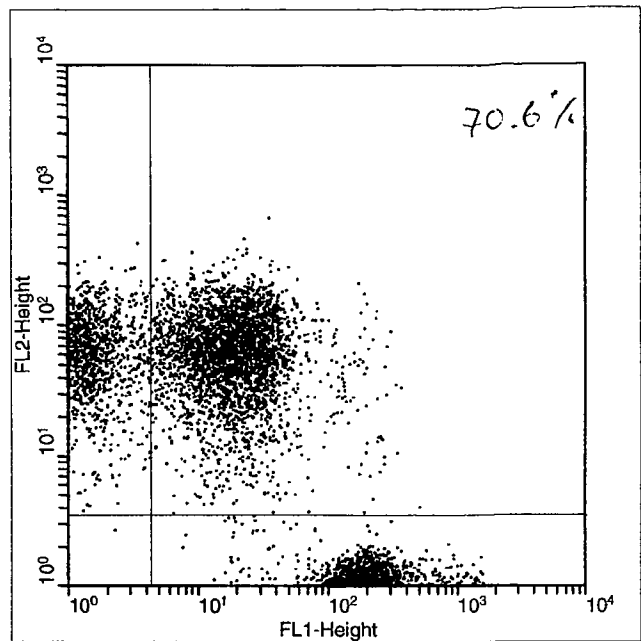
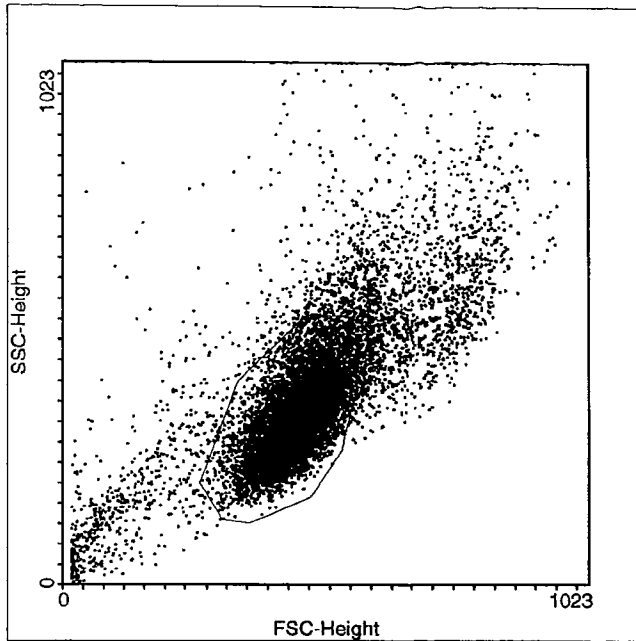
FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	62.9	1924	9.62	12.35
2 UR	19.8	55.6	5974	29.87	38.33
3 LL	1.8	2.5	1	0.01	0.01
4 LR	204.2	1.1	7685	38.43	49.31

} 50.68 → 75.6

A/D ~~DTA~~ = 1.03

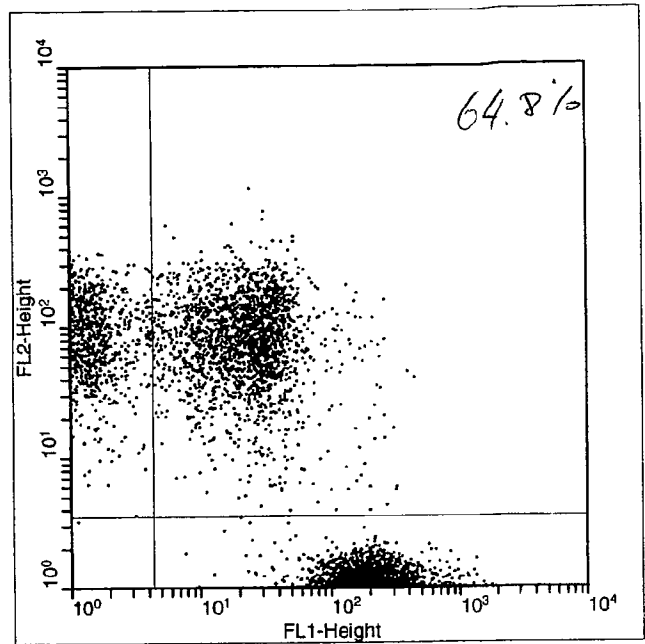
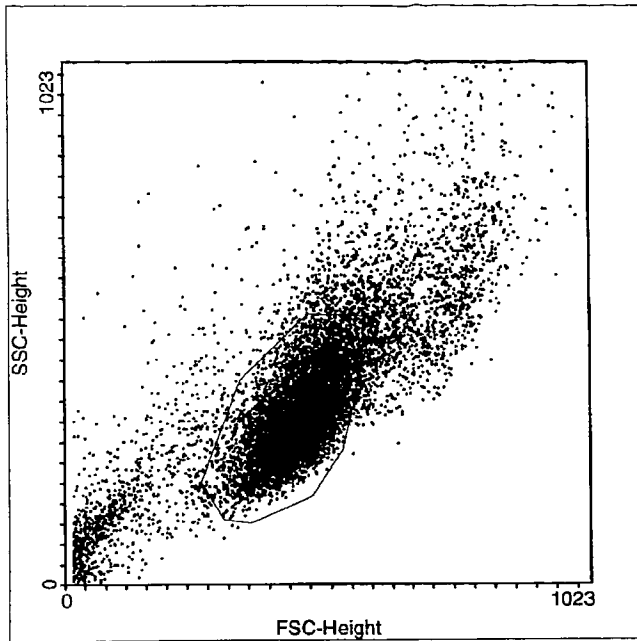


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 11:13:18 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: WB CA\*/DiI 9Gy 8h  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14968 74.84%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	61.0	2504	12.52	16.73
2 UR	18.8	57.0	6023	30.12	40.24
3 LL	2.3	2.7	1	0.01	0.01
4 LR	211.0	1.1	6440	32.20	43.03

} 56.97 → 70.6

A/D ~~DA~~ = 1.32

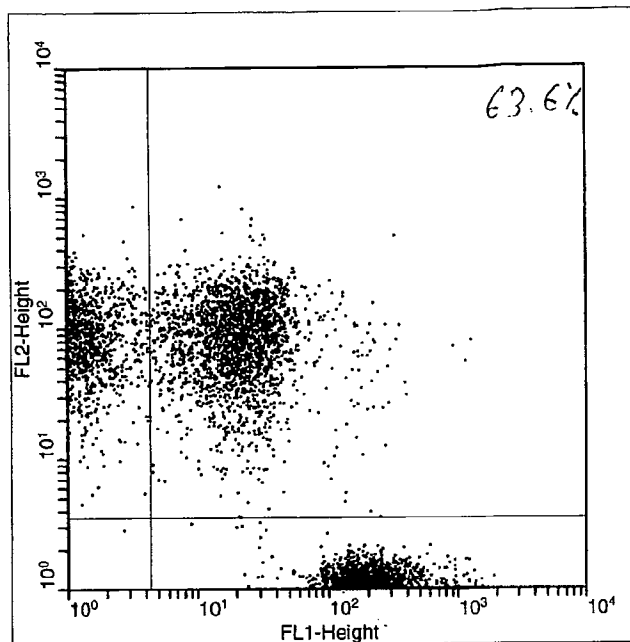
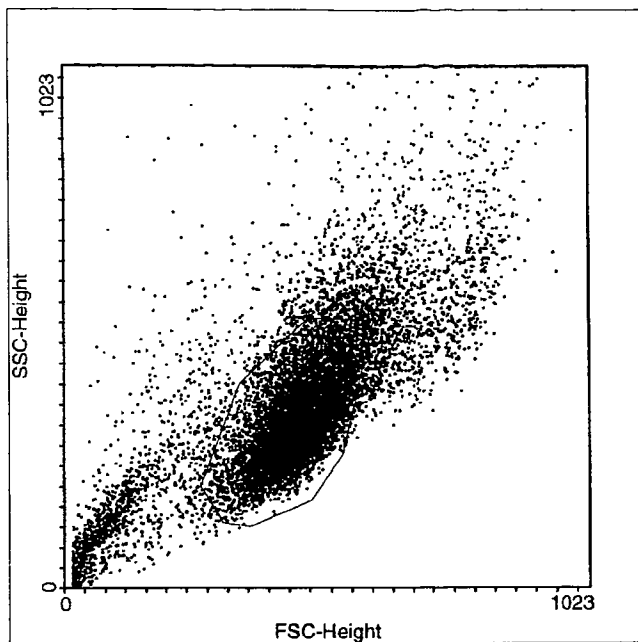


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 11:13:44 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: WB CA/DiI\* 9Gy 8h  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14455 72.28%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	88.9	2083	10.42	14.41
2 UR	22.4	76.9	3842	19.21	26.58
3 LL	1.1	3.1	2	0.01	0.01
4 LR	204.0	1.1	8528	42.64	59.00

} 40.99 → 64.8

A/D ~~B/A~~ = 0.69



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 17 11:23:44 2002  
 Gates: R1  
 Project: Experiment:  
 File: 041702 Sample: WB CA\*/DiI\* 9Gy 8h  
 Date: 17-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13750 68.75%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 164,140

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	89.6	2399	12.00	17.45
2 UR	21.1	78.1	4187	20.94	30.45
3 LL	1.7	2.6	4	0.02	0.03
4 LR	210.2	1.1	7160	35.80	52.07

47.9 → 63.6

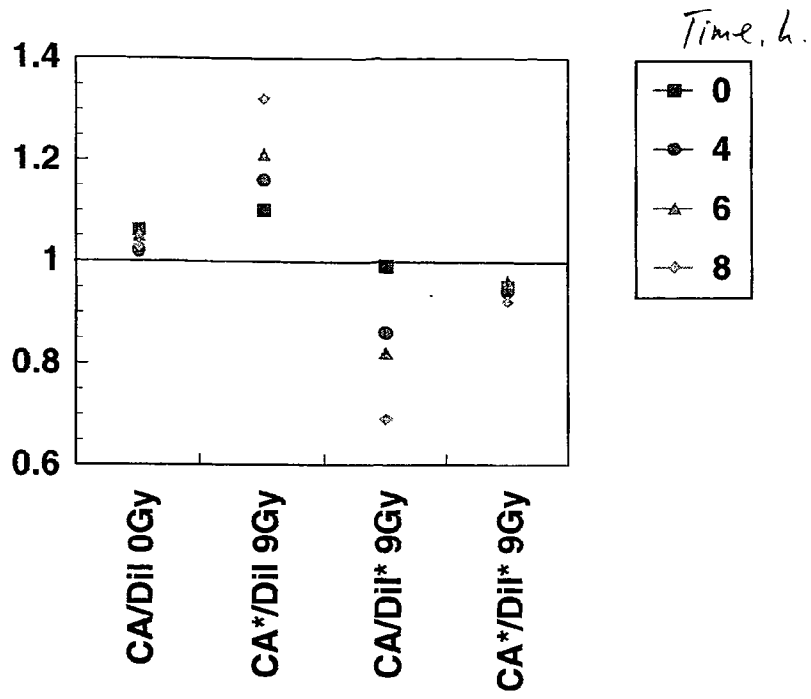
A/D ~~DA~~ = 0.92

## ACCEPTOR/DONOR RATION (AID)

APRIL 18, 2002

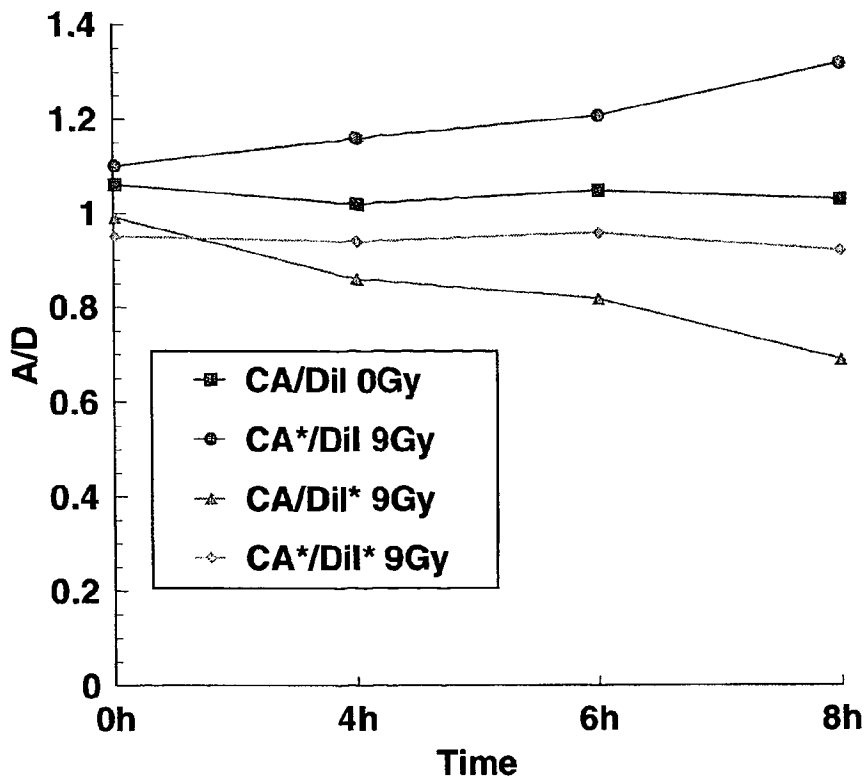
Label	A	B	C	D	E
	0 h	4 h	6 h	8 h	TIME
1 CA/DiI 0Gy	1.06	1.02	1.05	1.03	
2 A*/DiI 9Gy	1.1	1.16	1.21	1.32	
3 A/DiI* 9Gy	0.99	0.86	0.82	0.69	
4 A*/DiI* 9G	0.95	0.94	0.96	0.92	
5					

AID





Label	A	B	C	D	E
	CA/DiI	0GyA*/DiI	9GyA/DiI*	9GyA*/DiI*	9G
1	0h	1.06	1.1	0.99	0.95
2	4h	1.02	1.16	0.86	0.94
3	6h	1.05	1.21	0.82	0.96
4	8h	1.03	1.32	0.69	0.92
5					



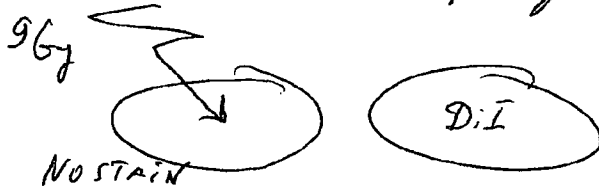
APRIL 17, 2002

APOPTOSIS ASSAY  
IRRADIATION (9 Gy) OF 50% POPULATION

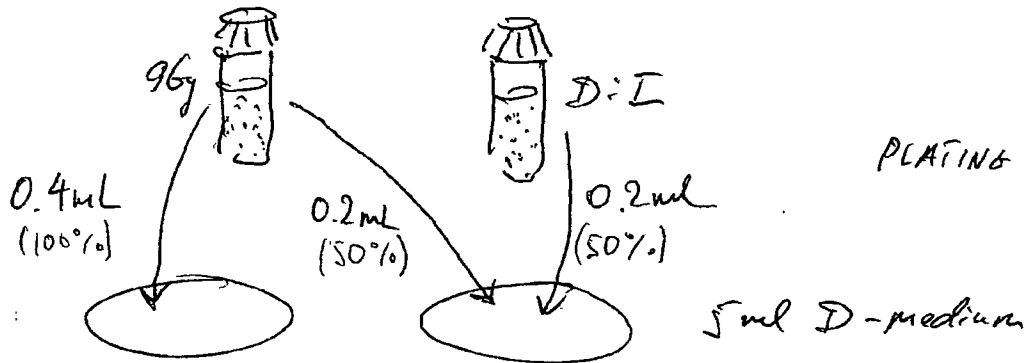
WBs - (31p)

IRRADIATION TIME: 8:30

100% confluency

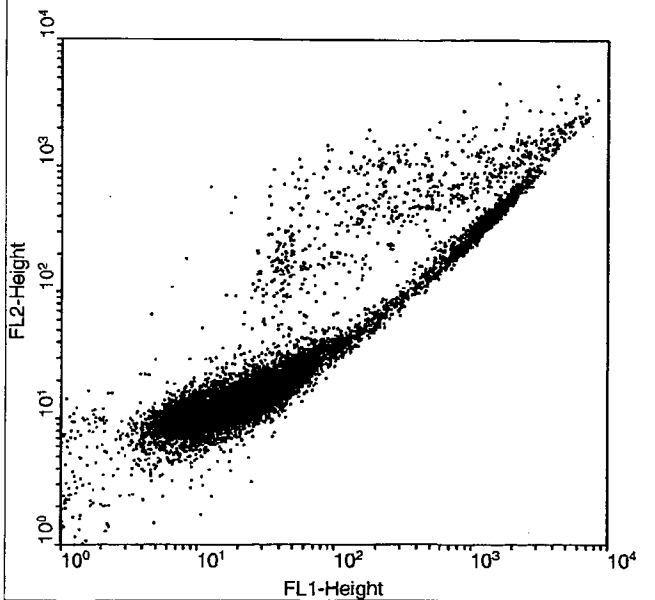
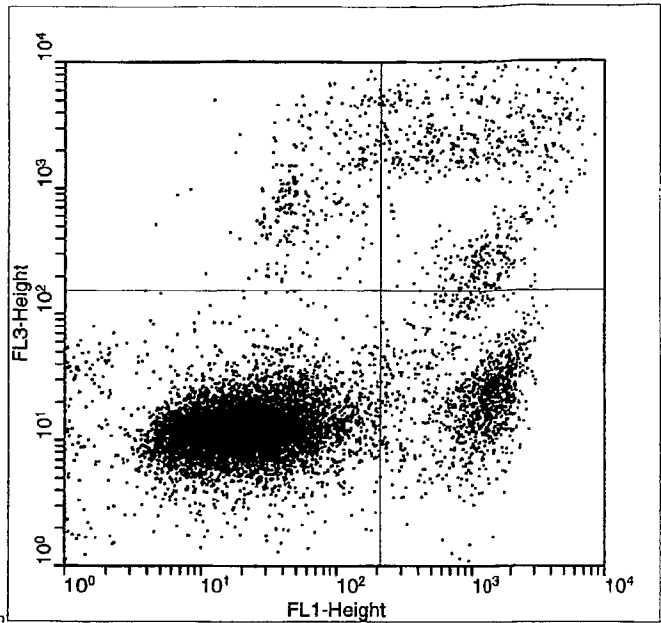
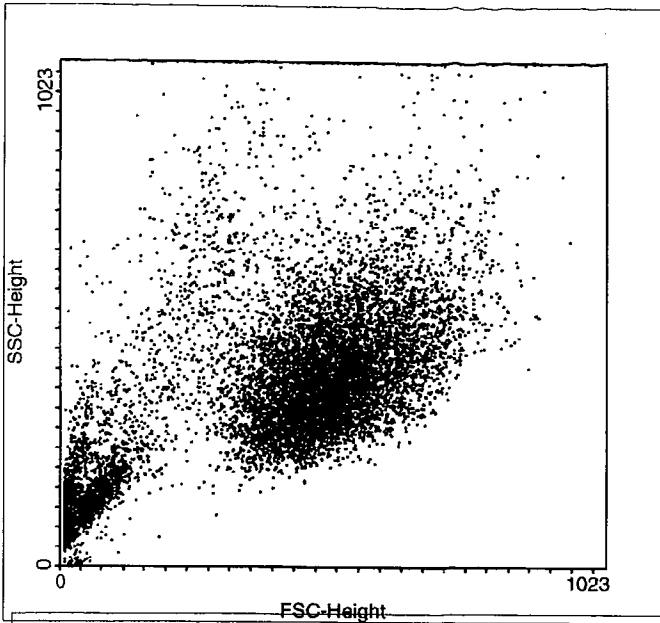


1 mL Trypsin + 2 mL D-medium



PLATING TIME:  
≈ 9:00

INCUBATION 0/NIGHT



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 18 10:22:46 2002

Gates:

Project: Experiment: WB 9Gy 24h

File: 041802 Sample: WB 9Gy 24h

Date: 18-Apr-2 Parameters: 7

Total Events 20000 Gated Events 20000 100.00%

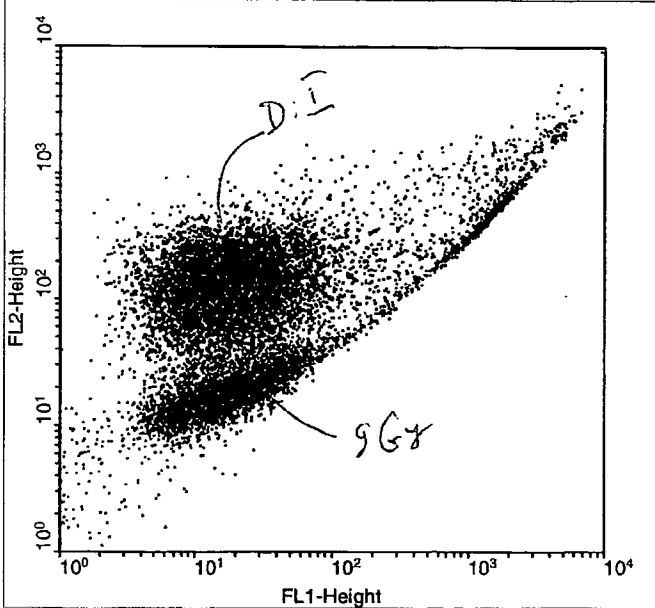
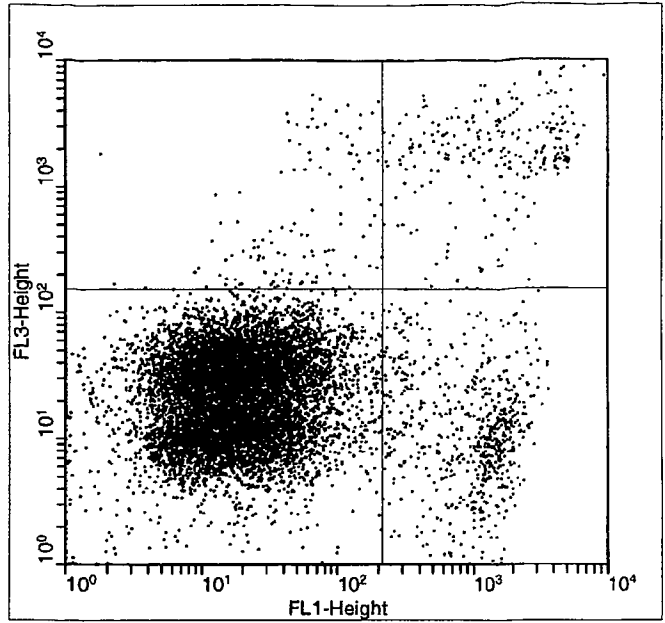
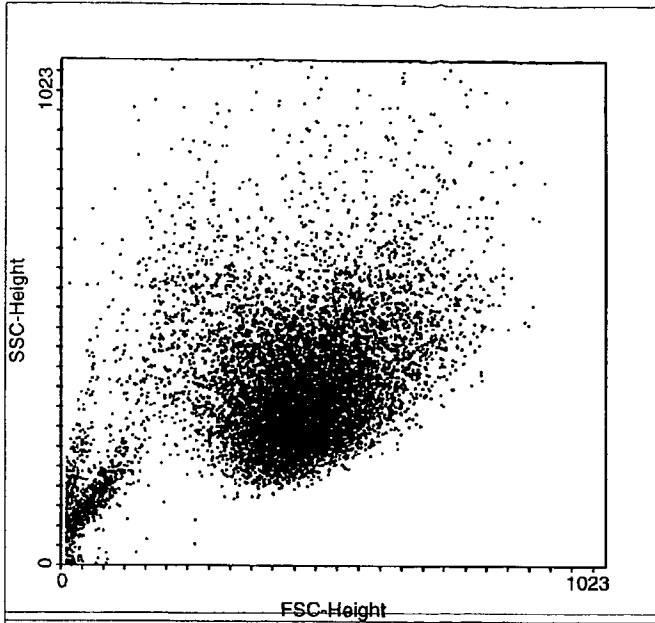
System: Log Parameter Means: Geometric

Quad Stats

FL1-Height(Log) vs FL3-Height(Log)

Quadrant x,y: 596,560

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	68.0	1140.4	575	2.88	2.88
2 UR	1191.3	1299.6	1232	6.16	6.16
3 LL	19.2	12.1	15998	79.99	79.99
4 LR	1056.8	22.2	2195	10.98	10.98



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 18 10:20:31 2002

Gates:

Project: Experiment: WB 9Gy/0Gy(DiI) 24h

File: 041802 Sample Parameters: 7

Date: 18-Apr-2 Total Events 20000 Gated Events 20000 100.00%

System: Log Parameter Means: Geometric

Quad Stats

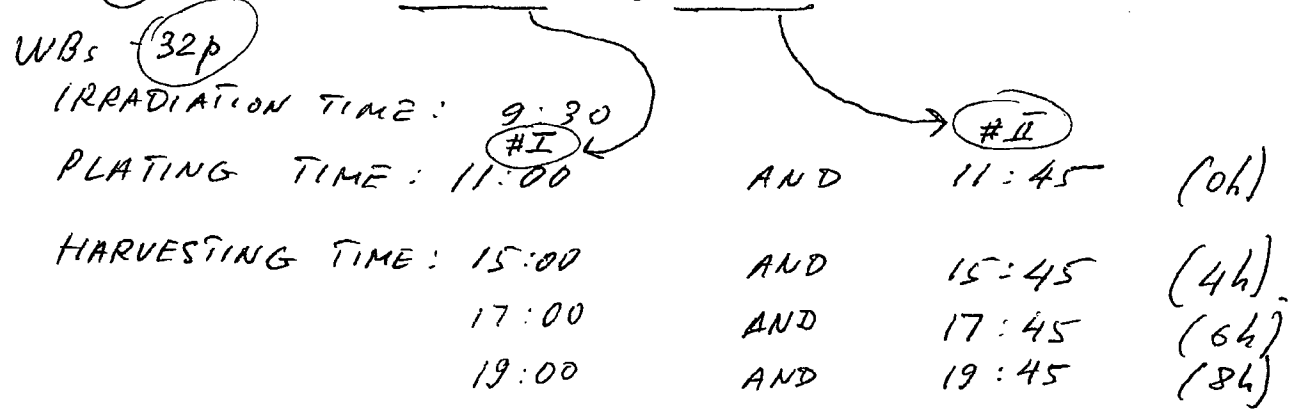
FL1-Height(Log) vs FL3-Height(Log)

Quadrant x,y: 608,504

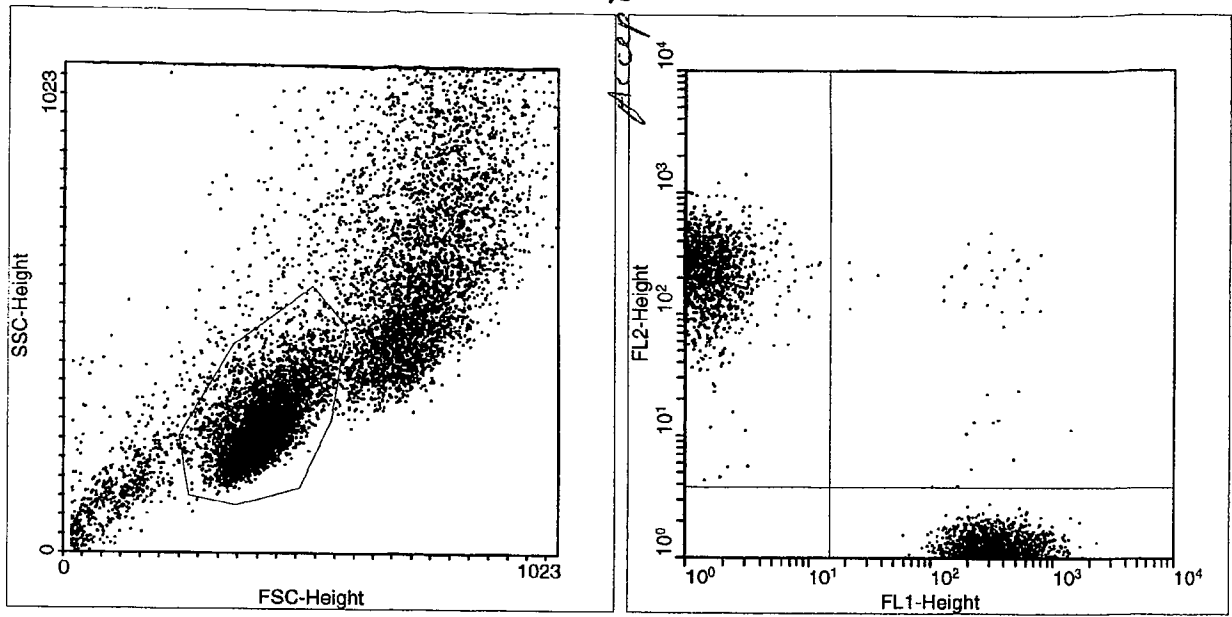
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	35.6	239.9	590	2.95	2.95
2 UR	1502.7	1516.2	549	2.75	2.75
3 LL	17.4	20.5	17667	88.33	88.33
4 LR	973.9	10.6	1194	5.97	5.97

APRIL 19.  
2002

- \* CELL-TO-CELL COMMUNICATION  
(50/50 - STAINING).
- \* IRRADIATION (96y) OF 50% POPULATION
- \* STAINING BEFORE AND AFTER IRRADIATION



NOTE: RESULTS ARE CONTROVERSIAL PROBABLY DUE TO ANALYSIS ON A THIRD DAY AFTER FIXATION.

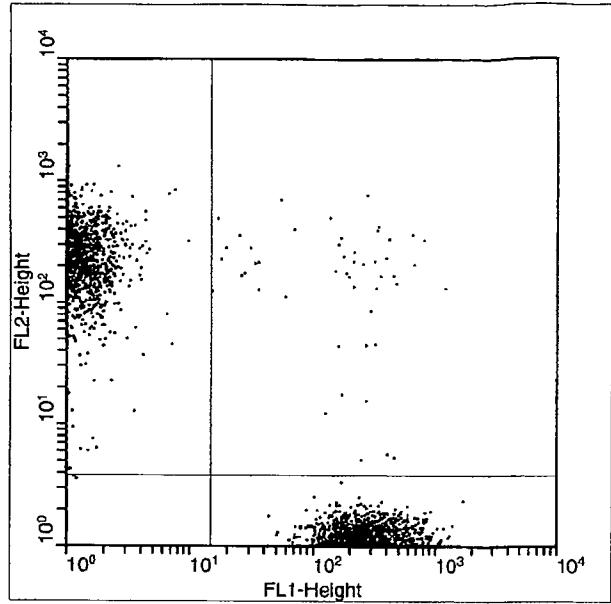
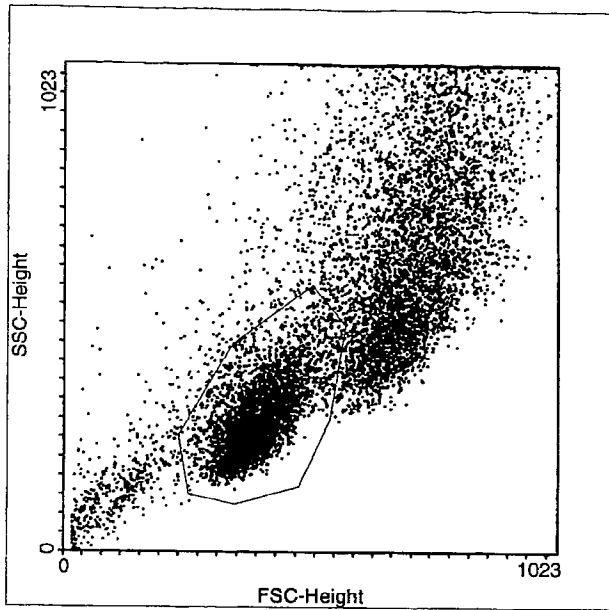


Donor

Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:27:22 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI 0Gy 0h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 9338 46.69%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 300,148

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	240.5	3686	18.43	39.47
2 UR	212.4	99.1	77	0.39	0.82
3 LL	1.0	2.2	2	0.01	0.02
4 LR	324.9	1.1	5573	27.87	59.68

AID = 0.66



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:30:17 2002

Gates: R1

Project: Experiment:

File: 042202 Sample: CA\*/DiI 9Gy 0h before

Date: 22-Apr-2 Parameters: 7

Total Events 20000 Gated Events 7516 37.58%

System: Log Parameter Means: Geometric

Quad Stats

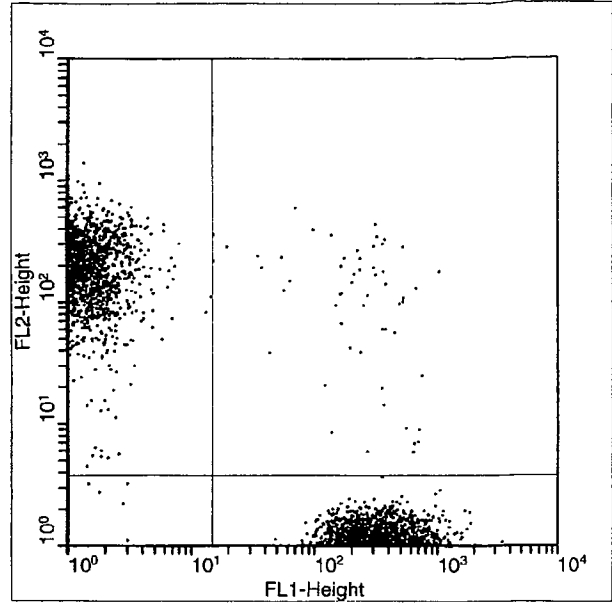
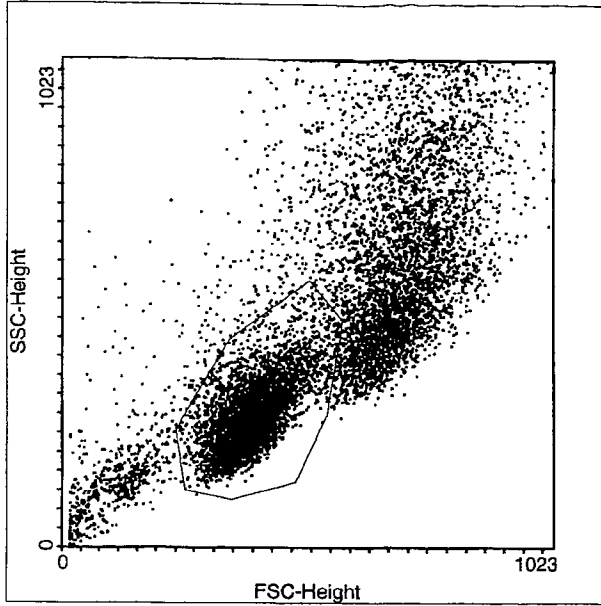
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 300,148

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.2	252.6	3443	17.22	45.81
2 UR	186.1	131.2	85	0.43	1.13
3 LL	1.1	3.5	3	0.02	0.04
4 LR	269.6	1.1	3985	19.93	53.02

$$A/D = 0.86$$

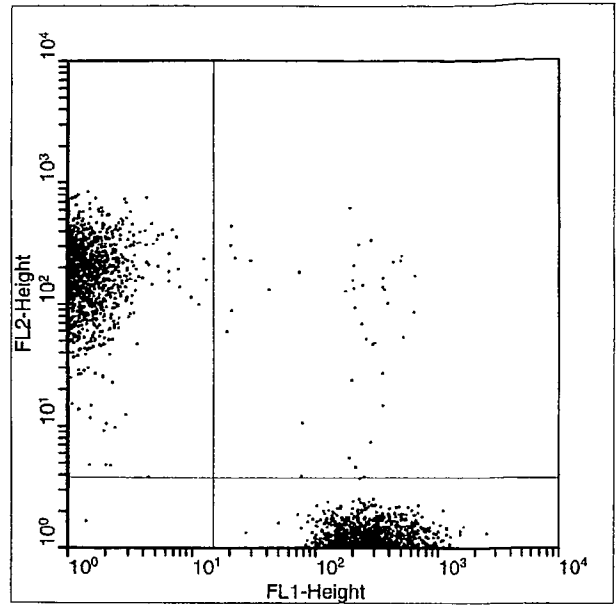
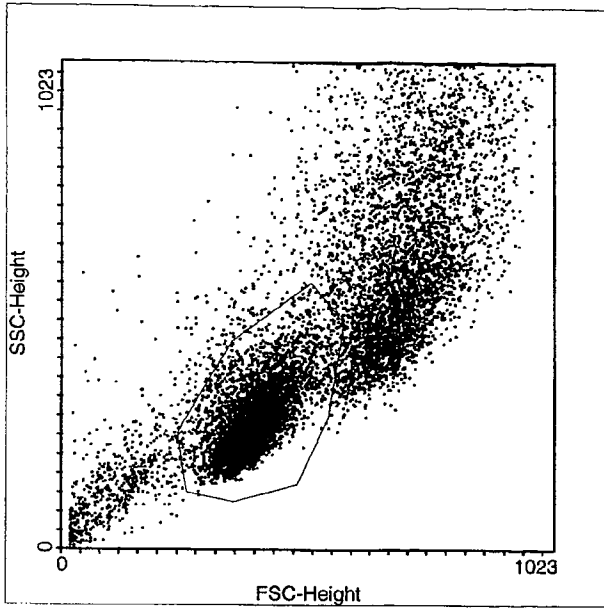




Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:30:58 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI\* 9Gy 0h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 8571 42.85%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 300,148  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	199.2	3591	17.96	41.90
2 UR	219.6	94.9	106	0.53	1.24
3 LL	1.9	2.6	10	0.05	0.12
4 LR	316.8	1.1	4864	24.32	56.75

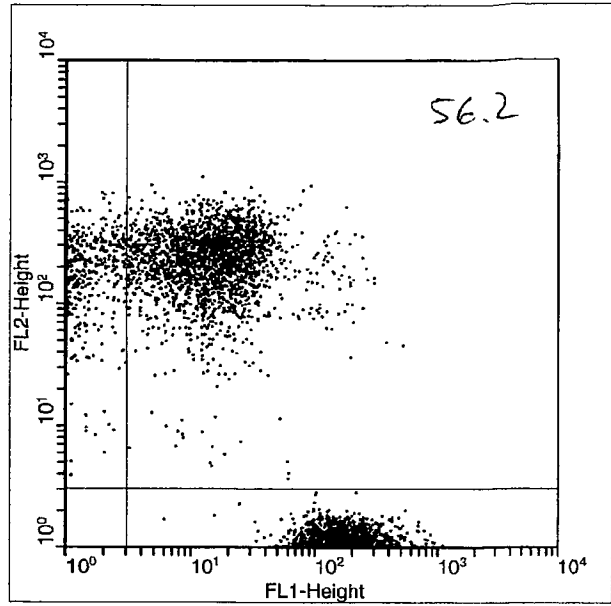
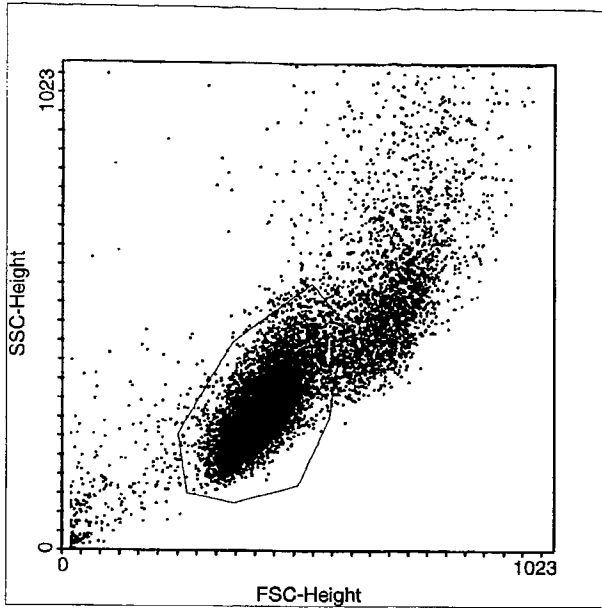
$$A_{1D} = 0.74$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:31:26 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/DiI\* 9Gy 0h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 8917 44.58%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 300,148  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.2	199.3	4371	21.86	49.02
2 UR	166.3	72.4	76	0.38	0.85
3 LL	1.2	2.4	2	0.01	0.02
4 LR	271.3	1.1	4468	22.34	50.11

AID = 0.98



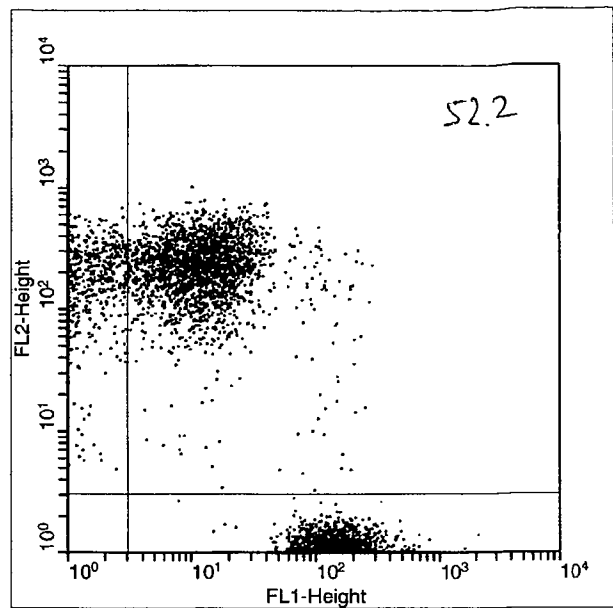
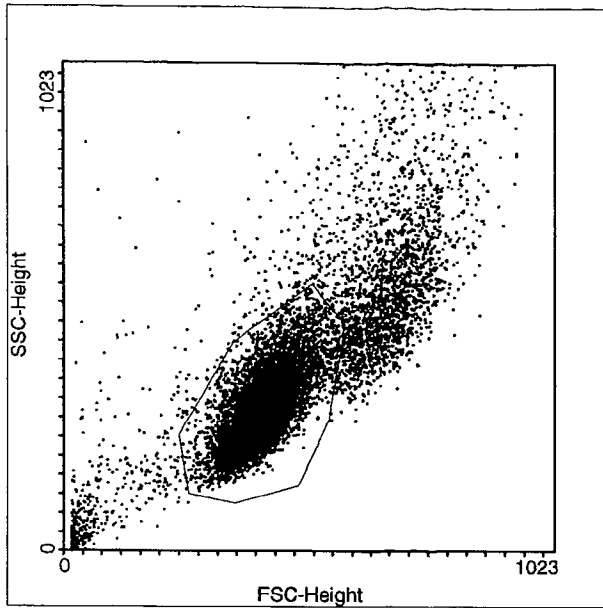
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:40:54 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI 0Gy 4h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14207 71.04%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	218.2	3239	16.20	22.80
2 UR	15.2	214.0	4156	20.78	29.25
3 LL	0.0	0.0	0	0.00	0.00
4 LR	170.1	1.1	6812	34.06	47.95

} 52.05 → 56.2

A/D = 1.09



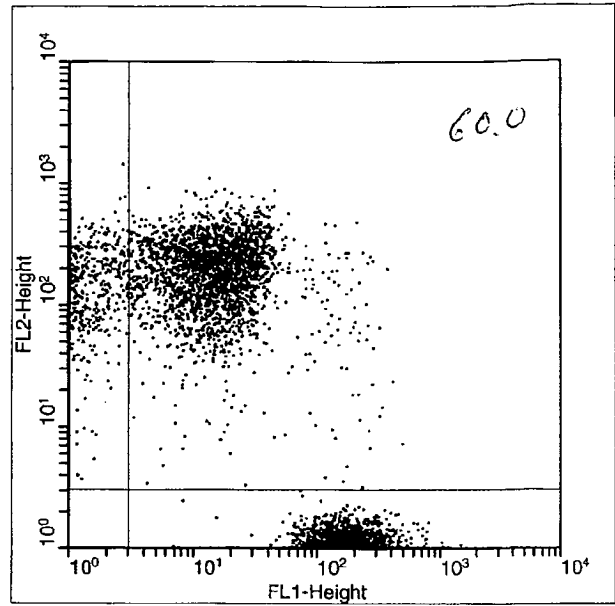
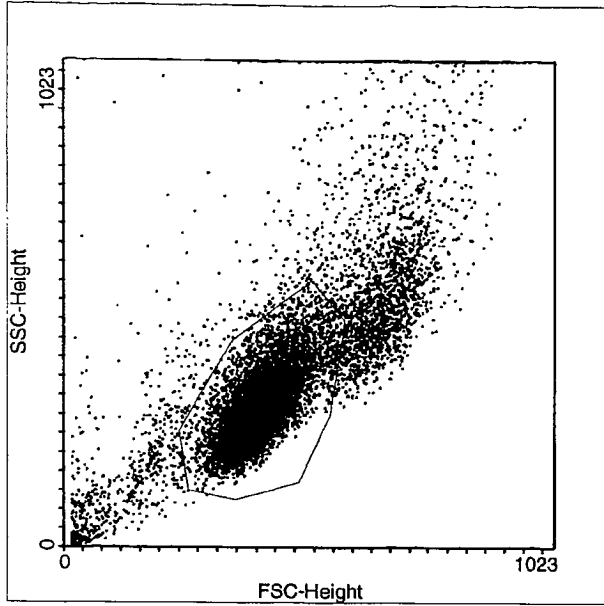
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:41:46 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/DiI 9Gy 4h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14769 73.85%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	214.7	3790	18.95	25.66
2 UR	12.6	213.9	4146	20.73	28.07
3 LL	0.0	0.0	0	0.00	0.00
4 LR	149.5	1.1	6833	34.17	46.27

53.73 → 52.2

A/D = 1.16



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:42:38 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI\* 9Gy 4h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14740 73.70%  
 System: Log Parameter Means: Geometric  
 Quad Stats

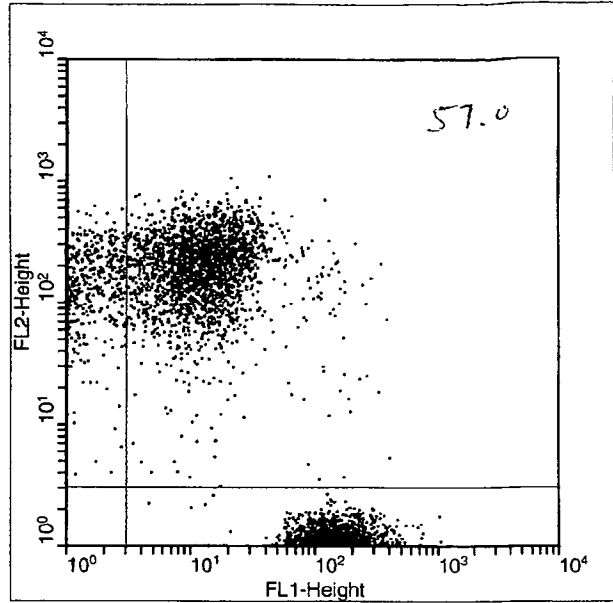
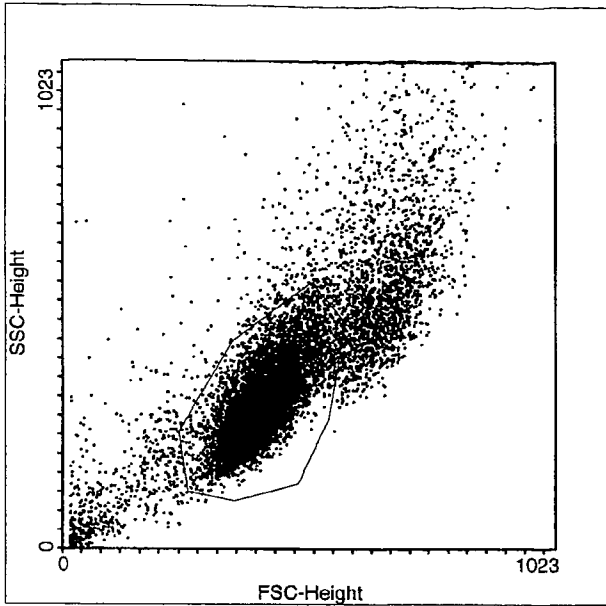
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x, y: 124, 124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	181.7	3105	15.53	21.07
2 UR	15.5	182.6	4667	23.34	31.66
3 LL	0.0	0.0	0	0.00	0.00
4 LR	174.2	1.1	6968	34.84	47.27

} 52.73 → 60.0

A/D = 1.12



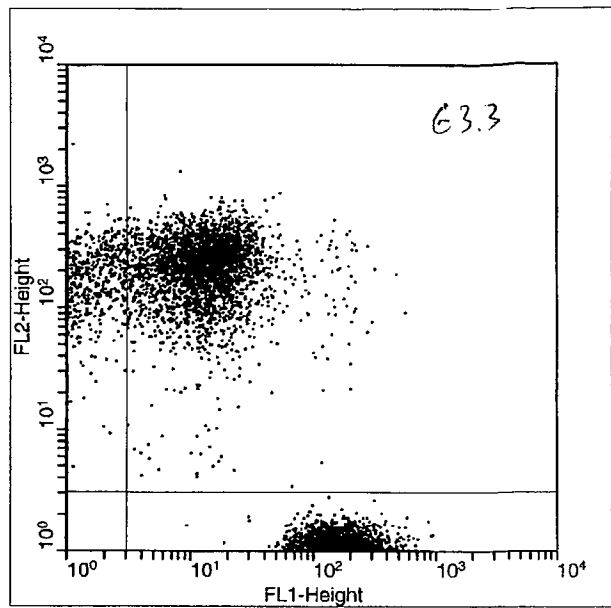
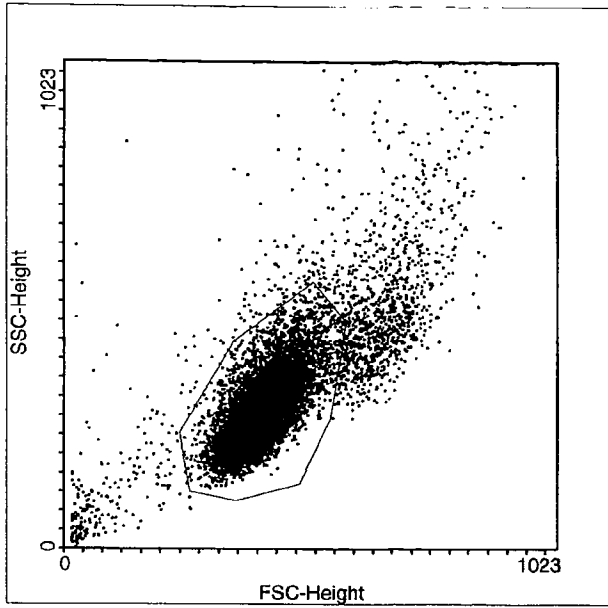
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:43:12 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/DiI\* 9Gy 4h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14540 72.70%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	180.4	3363	16.82	23.13
2 UR	13.0	185.2	4454	22.27	30.63
3 LL	1.1	2.8	2	0.01	0.01
4 LR	150.5	1.1	6721	33.61	46.22

53.76 → 57.0

A/D = 1.16



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:43:49 2002

Gates: R1

Project: Experiment:

File: 042202 Sample: CA/DiI 0Gy 6h before

Date: 22-Apr-2 Parameters: 7

Total Events 20000 Gated Events 17104 85.52%

System: Log Parameter Means: Geometric

Quad Stats

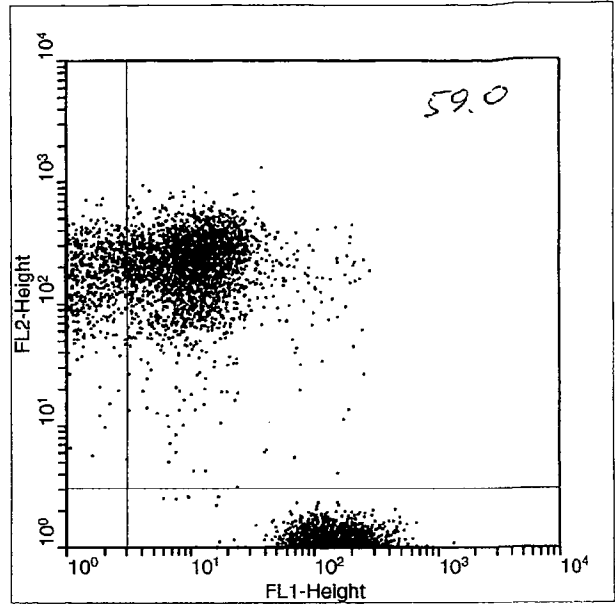
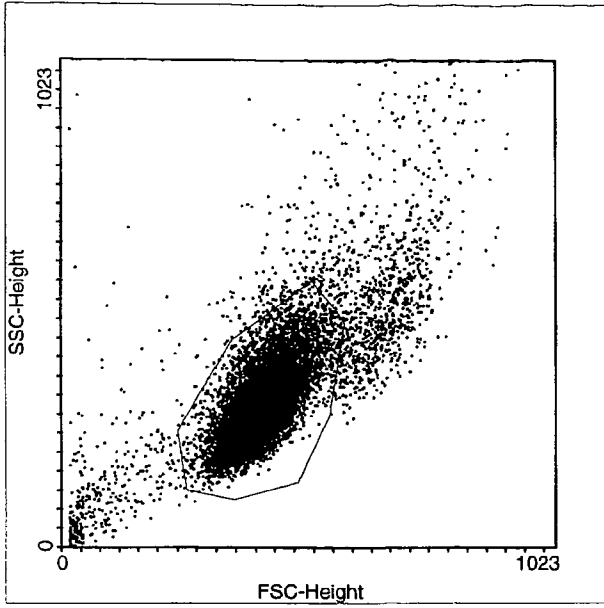
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.2	206.0	3312	16.56	19.36
2 UR	13.3	202.5	5710	28.55	33.38
3 LL	0.0	0.0	0	0.00	0.00
4 LR	160.0	1.1	8082	40.41	47.25

52.74 → 63.3

AID = 1.12



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:44:18 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/DiI 9Gy 6h before  
 Date: 22-Apr-2 Parameters: /  
 Total Events 20000 Gated Events 16963 84.82%  
 System: Log Parameter Means: Geometric  
 Quad Stats

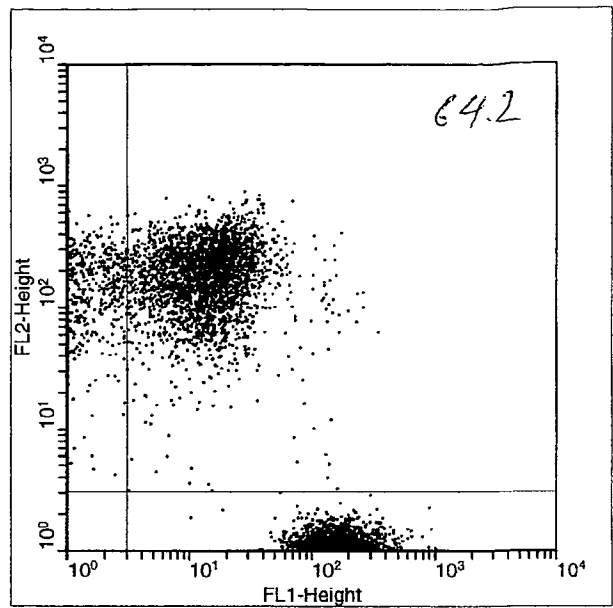
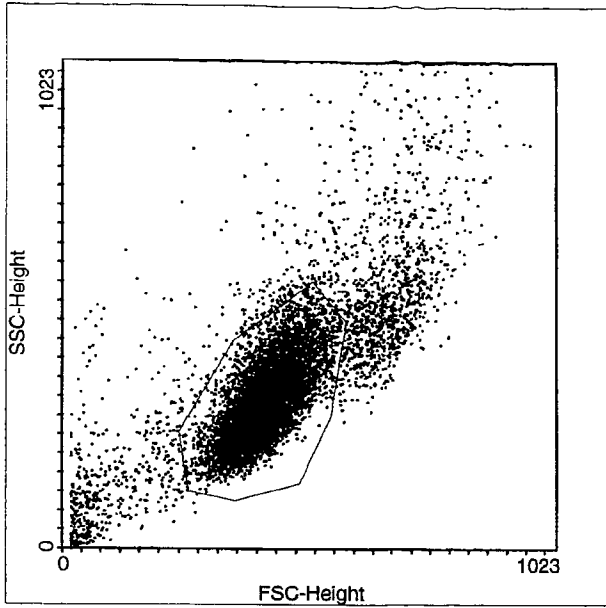
FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	206.9	3742	18.71	22.06
2 UR	11.2	201.6	5390	26.95	31.78
3 LL	1.0	1.1	1	0.01	0.01
4 LR	143.6	1.1	7830	39.15	46.16

53.84 → 59.0

A/D = 1.17



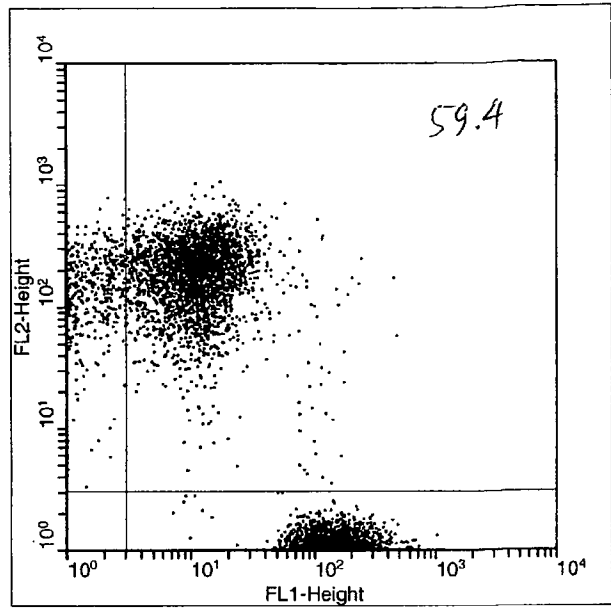
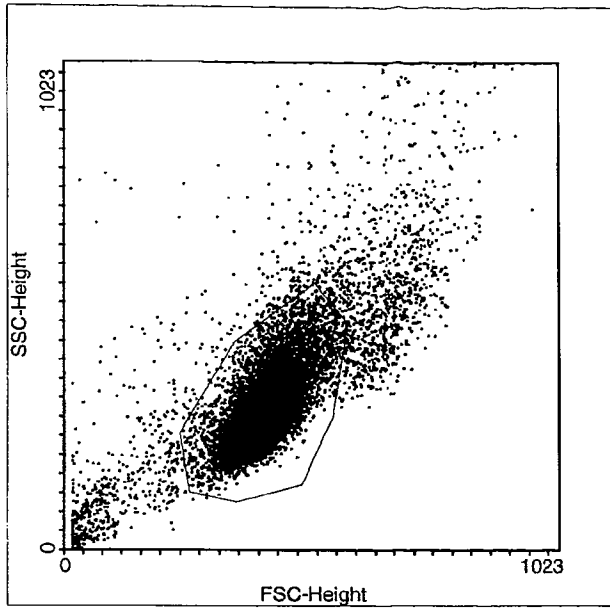


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:45:05 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI\* 9Gy 6h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16448 82.24%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	174.2	3029	15.15	18.42
2 UR	14.0	169.9	5423	27.12	32.97
3 LL	1.0	2.4	2	0.01	0.01
4 LR	165.3	1.1	7994	39.97	48.60

} 51.39 → 64.2

A/D = 1.06



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:45:28 2002  
 Gates: R1

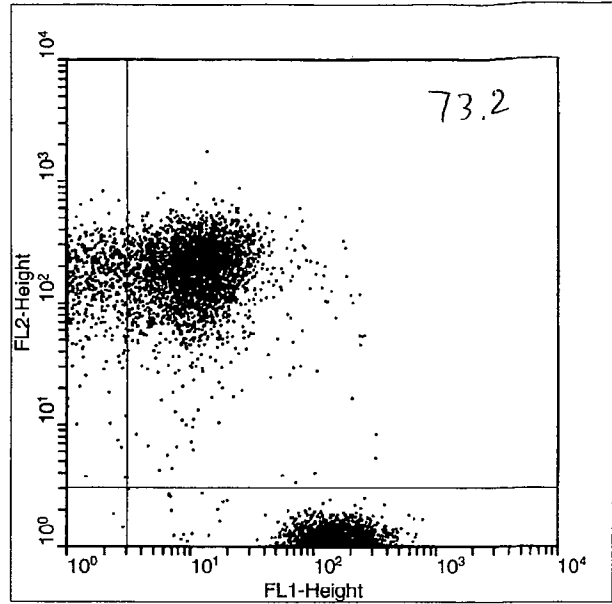
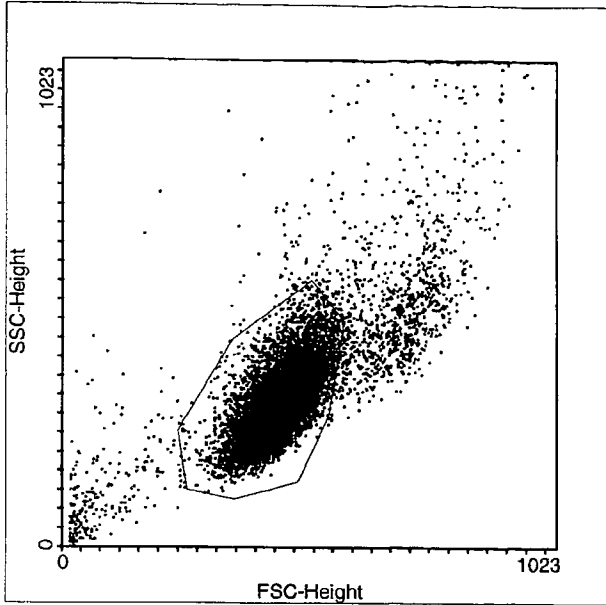
Project: Experiment:  
 File: 042202 Sample: CA\*/DiI\* 9Gy 6h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16520 82.60%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	172.7	3610	18.05	21.85
2 UR	11.6	172.2	5285	26.43	31.99
3 LL	1.0	2.1	1	0.01	0.01
4 LR	143.0	1.1	7624	38.12	46.15

} 53.84 → 59.4

A/D = 1.17



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:46:01 2002

Gates: R1

Project: Experiment:

File: 042202 Sample: CA/DiI 0Gy 8h before

Date: 22-Apr-2 Parameters: 7

Total Events 20000 Gated Events 17395 86.97%

System: Log Parameter Means: Geometric

Quad Stats

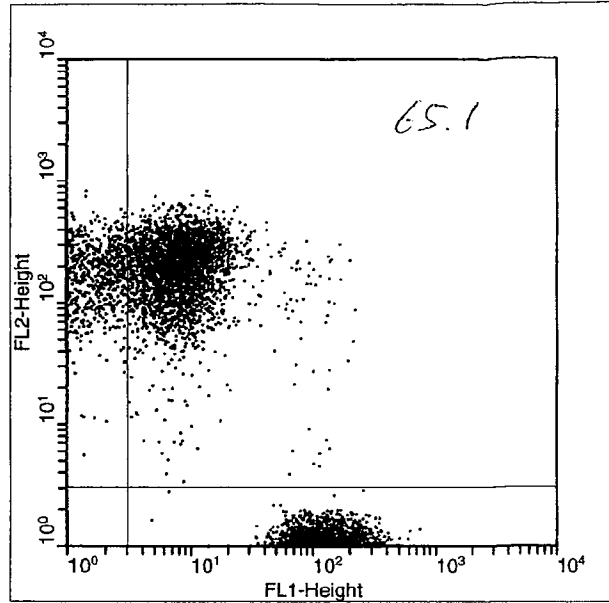
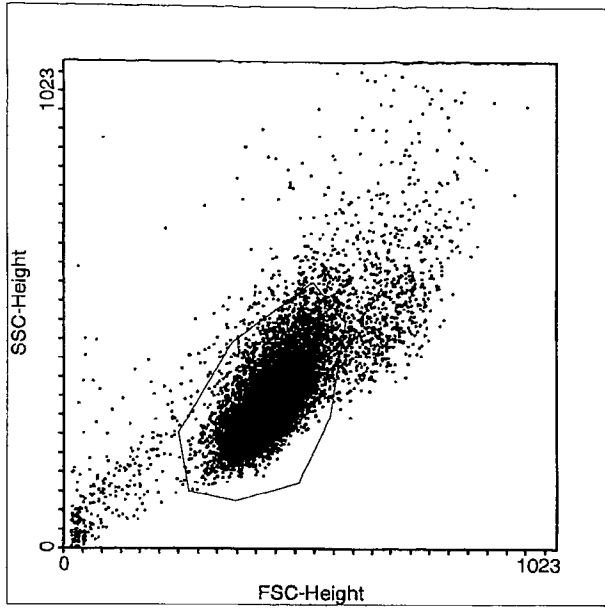
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.2	181.0	2412	12.06	13.87
2 UR	11.2	179.4	6592	32.96	37.90
3 LL	2.6	2.0	3	0.02	0.02
4 LR	154.1	1.1	8388	41.94	48.22

51.77 → 73.2

A/D → 1.07



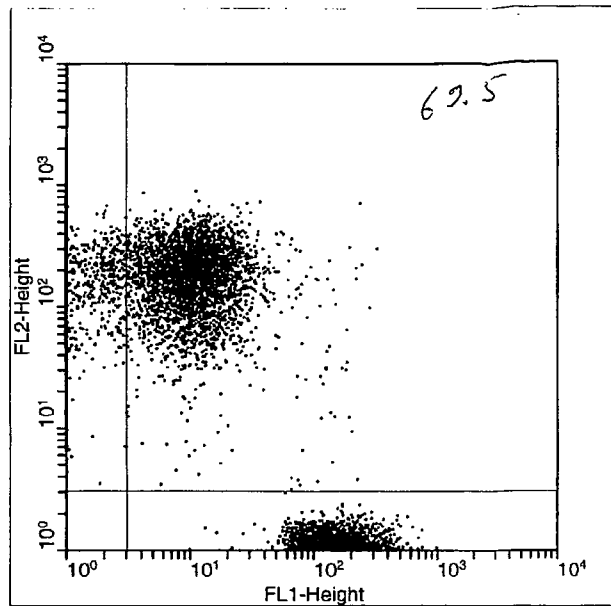
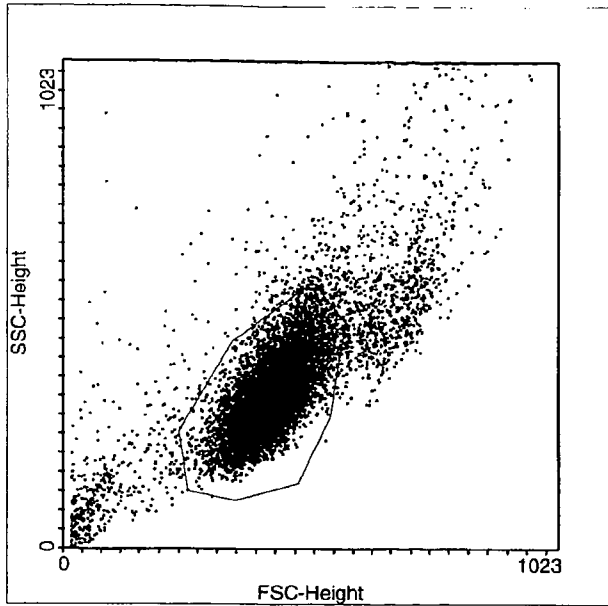
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:46:37 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/DII 9Gy 8h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 17402 87.01%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.2	192.5	3334	16.67	19.16
2 UR	8.6	186.1	6224	31.12	35.77
3 LL	0.0	0.0	0	0.00	0.00
4 LR	121.8	1.1	7844	39.22	45.08

54.93 → 65.1

$$A/D = 1.22$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:47:09 2002

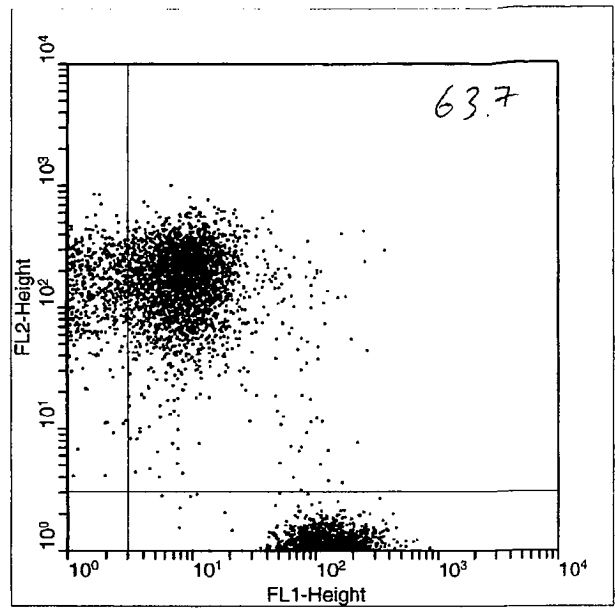
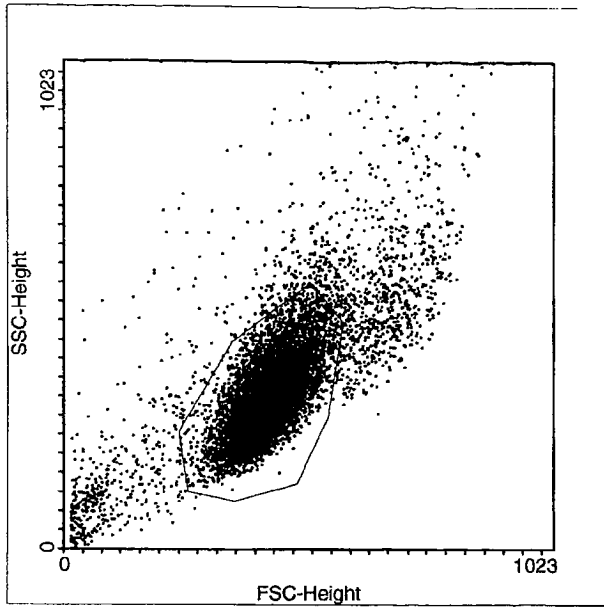
Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI\* 9Gy 8h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16891 84.46%

System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.2	166.6	2608	13.04	15.44
2 UR	11.2	162.0	5953	29.77	35.24
3 LL	1.0	2.8	1	0.01	0.01
4 LR	148.1	1.1	8329	41.65	49.31

50.62 → 69.5

AID = 1.03



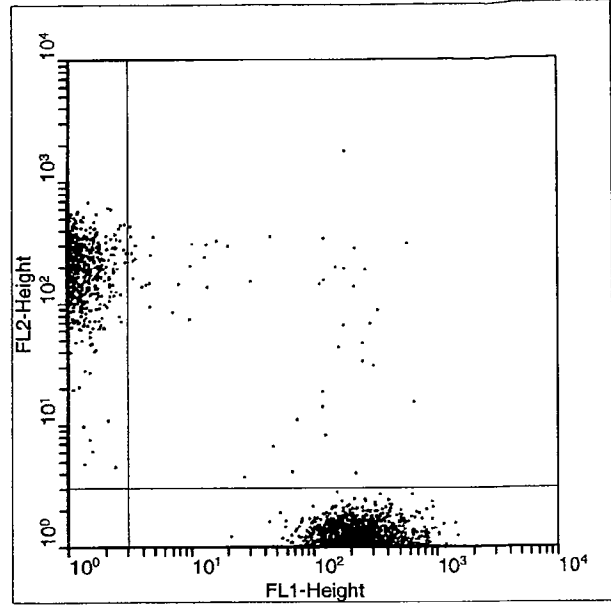
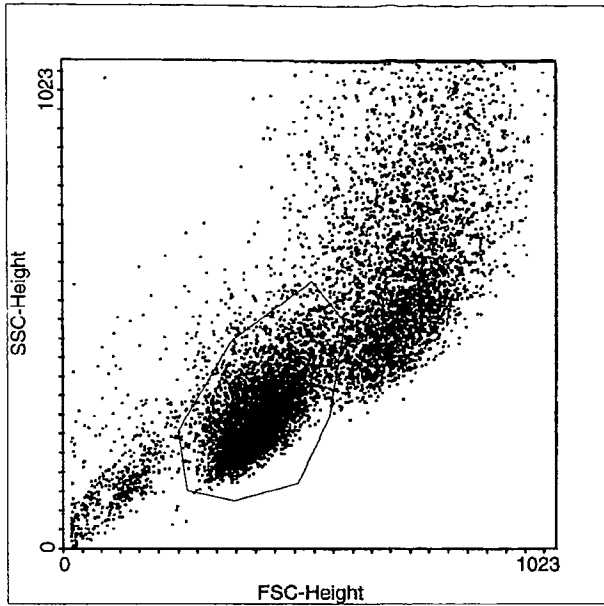
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:47:45 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/DiI\* 9Gy 8h before  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16955 84.78%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.2	168.5	3291	16.46	19.41
2 UR	9.1	158.7	5787	28.94	34.13
3 LL	0.0	0.0	0	0.00	0.00
4 LR	125.4	1.1	7877	39.39	46.46

53.54 → 63.7

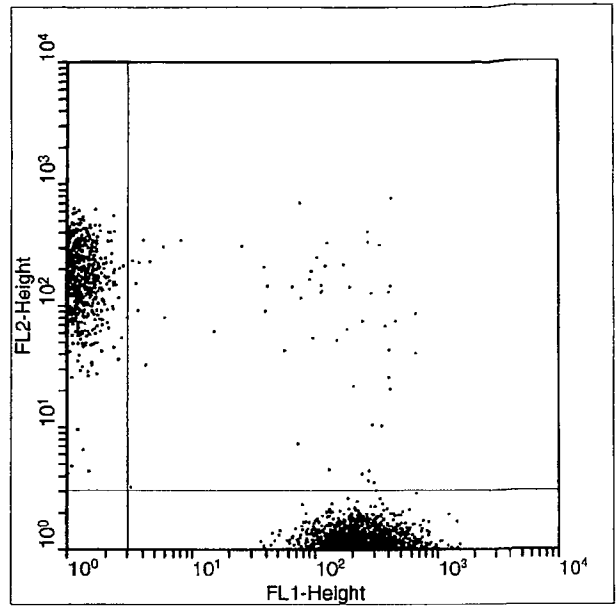
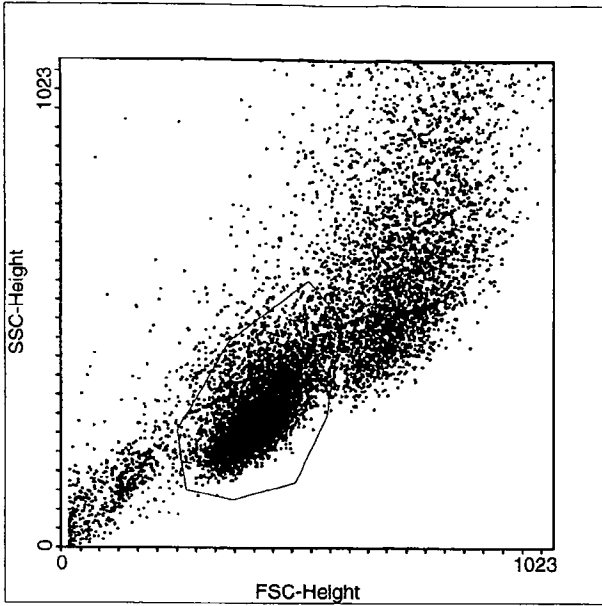
A/D = 1.15



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:48:22 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI 0Gy 0h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 10020 50.10%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	217.9	3786	18.93	37.78
2 UR	47.5	91.9	96	0.48	0.96
3 LL	0.0	0.0	0	0.00	0.00
4 LR	231.9	1.1	6138	30.69	61.26

$A/D = 0.62$

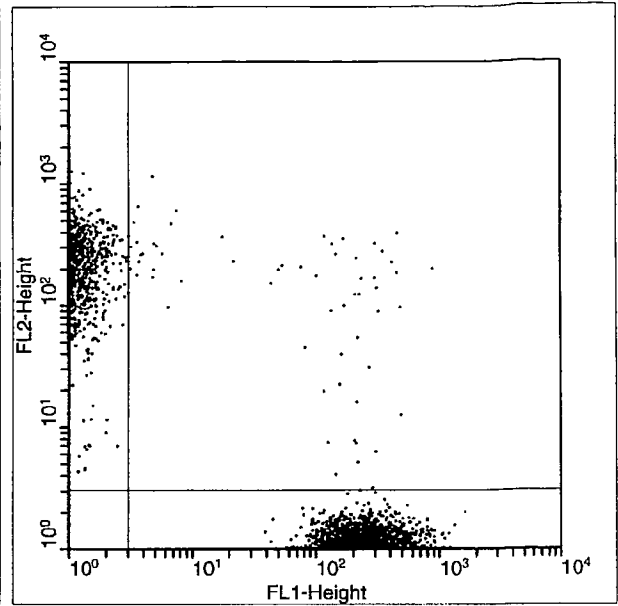
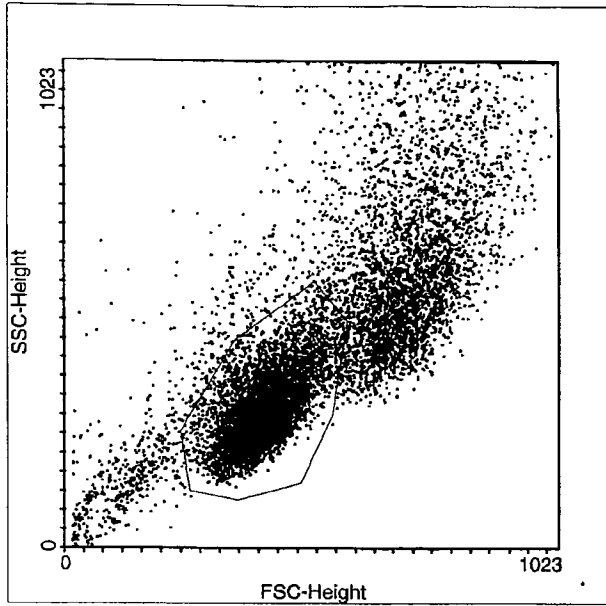


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:48:51 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/DiI 9Gy 0h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 10392 51.96%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	214.5	3968	19.84	38.18
2 UR	72.4	81.7	109	0.55	1.05
3 LL	1.0	2.1	1	0.01	0.01
4 LR	230.2	1.1	6314	31.57	60.76

*AID = 0.63*

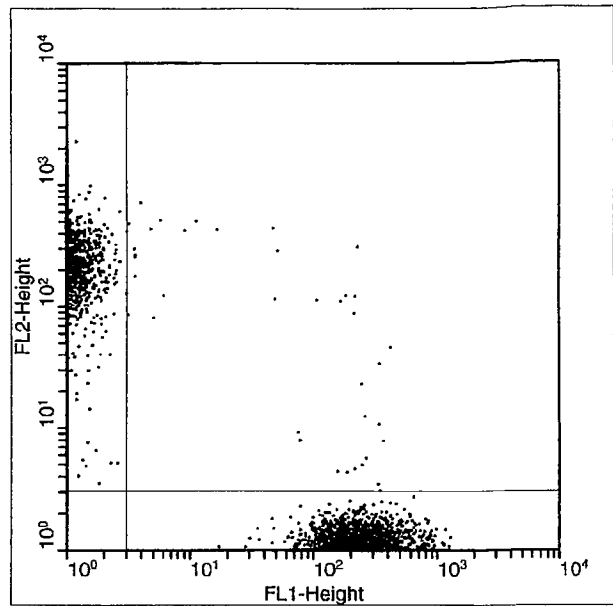
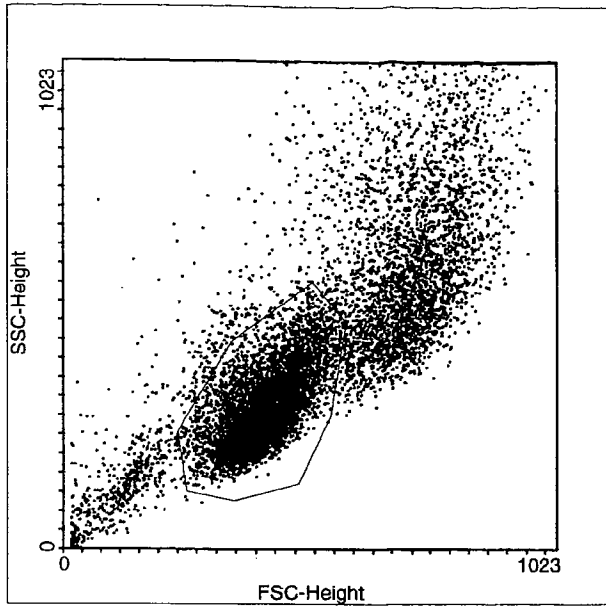




Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:49:12 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample CA/D11\* 9Gy 0h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 11101 55.51%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	256.1	5290	26.45	47.65
2 UR	51.8	81.4	126	0.63	1.14
3 LL	1.0	2.1	3	0.02	0.03
4 LR	245.1	1.1	5682	28.41	51.18

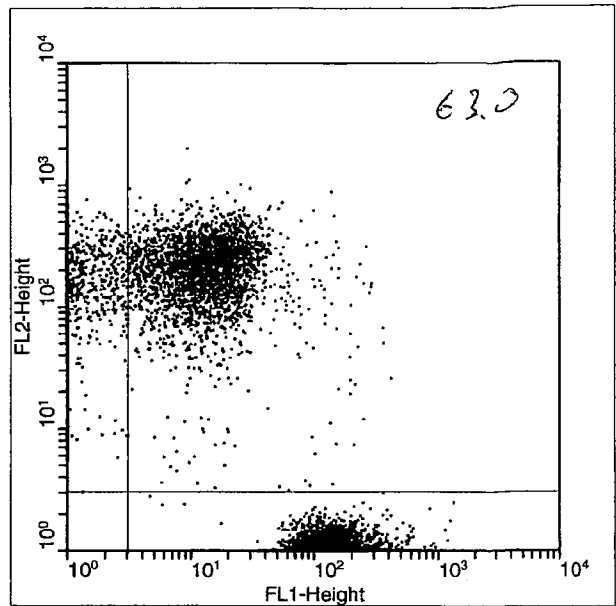
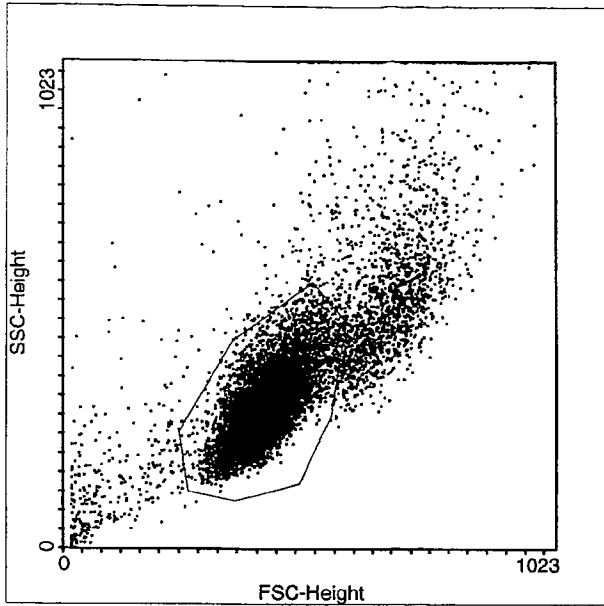
$A/D = 0.93$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:49:38 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/DiI\* 9Gy 0h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 11462 57.31%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	257.8	5306	26.53	46.29
2 UR	52.5	83.7	104	0.52	0.91
3 LL	2.0	2.1	2	0.01	0.02
4 LR	239.2	1.1	6050	30.25	52.78

*AID = 0.88*



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:50:04 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI 0Gy 4h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 15989 79.94%  
 System: Log Parameter Means: Geometric

Quad Stats

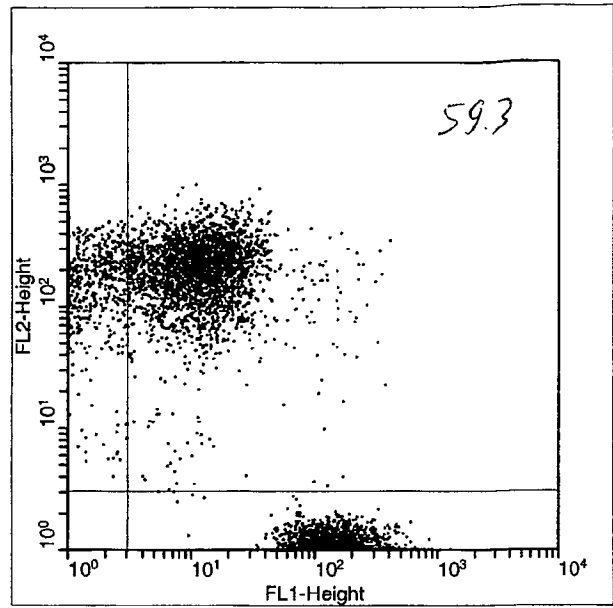
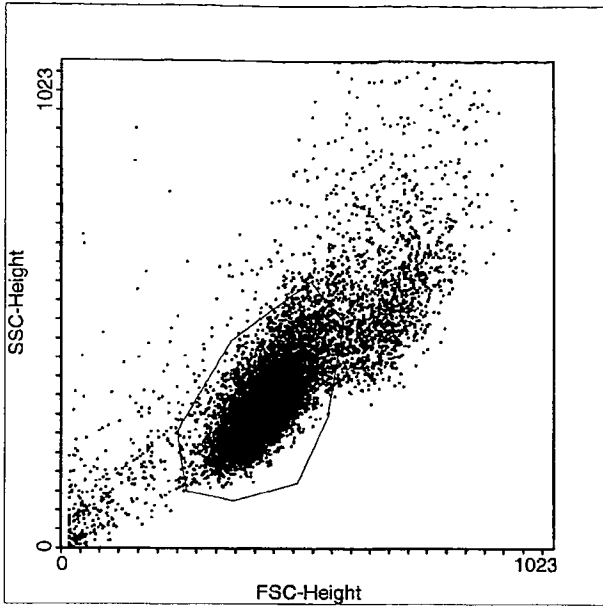
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	183.9	3015	15.08	18.86
2 UR	13.0	184.4	5126	25.63	32.06
3 LL	0.0	0.0	0	0.00	0.00
4 LR	140.3	1.1	7848	39.24	49.08

} 50.92 → 63.0

A/D = 1.04



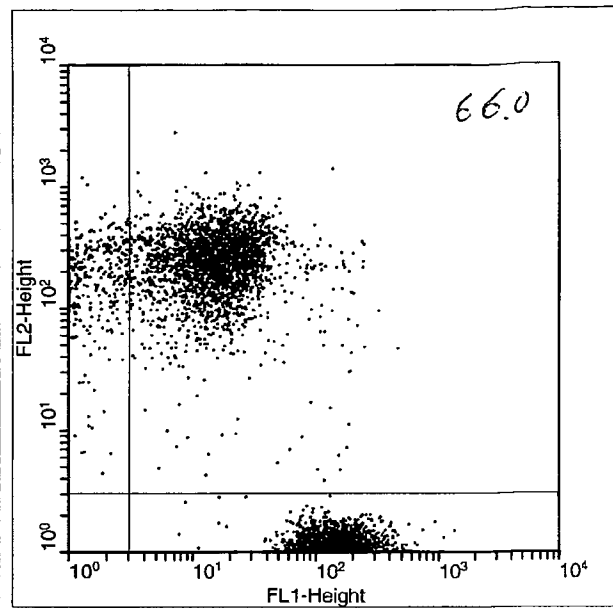
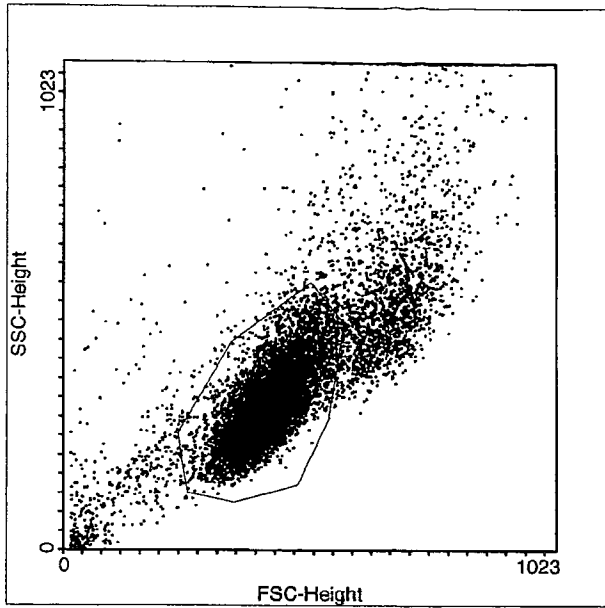
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:50:33 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/DI1 9Gy 4h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16162 80.81%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	184.5	3640	18.20	22.52
2 UR	12.0	187.1	5308	26.54	32.84
3 LL	0.0	0.0	0	0.00	0.00
4 LR	136.1	1.1	7214	36.07	44.64

} 55.36 → 59.3

AID = 1.24



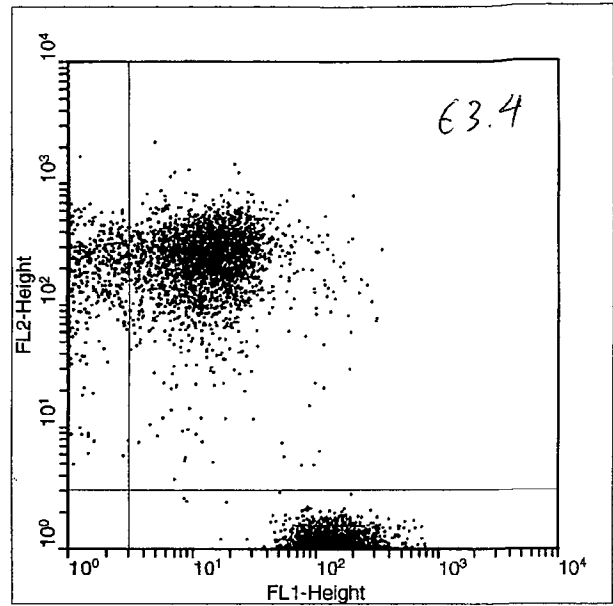
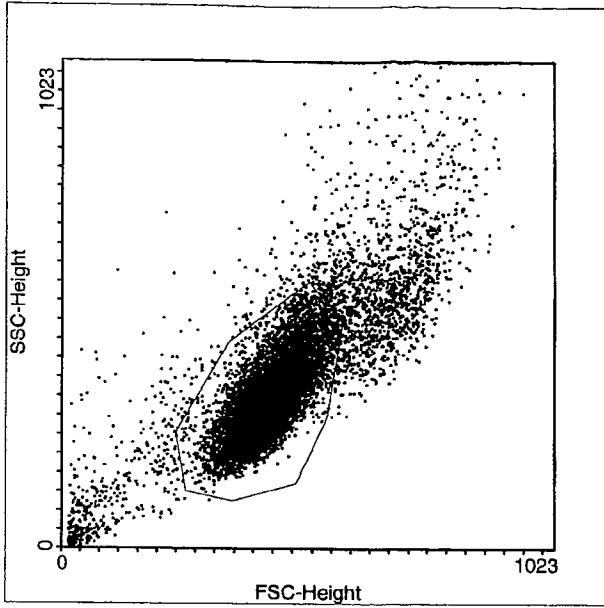
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:50:54 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI\* 9Gy 4h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 15649 78.25%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	227.4	2448	12.24	15.64
2 UR	15.5	230.3	4749	23.75	30.35
3 LL	0.0	0.0	0	0.00	0.00
4 LR	143.6	1.1	8452	42.26	54.01

45.99 → 66.0

AID = 0.85

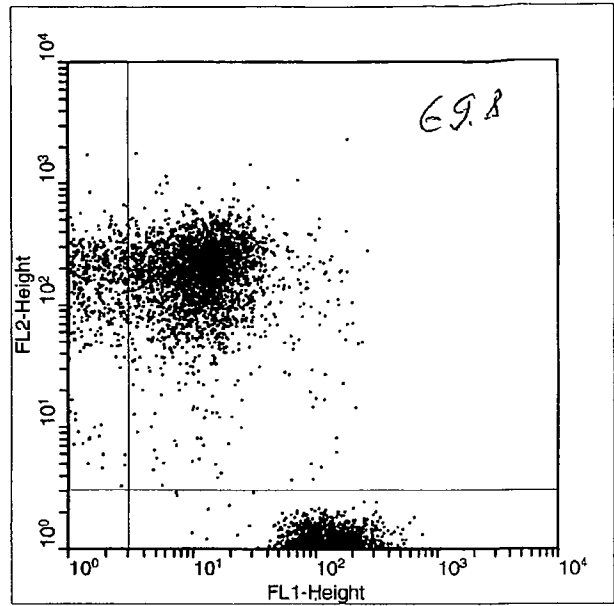
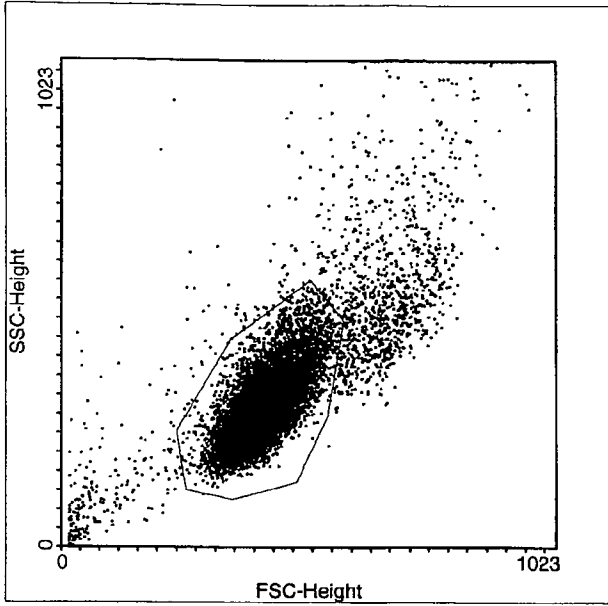


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:51:20 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/DII\* 9Gy 4h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16072 80.36%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	226.3	2985	14.93	18.57
2 UR	13.6	228.9	5172	25.86	32.18
3 LL	1.1	2.2	1	0.01	0.01
4 LR	136.4	1.1	7914	39.57	49.24

50.75 → 63.4

AID = 1.03



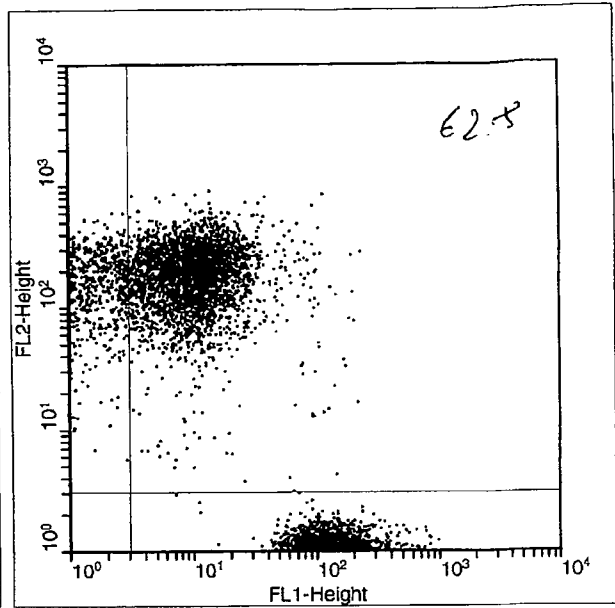
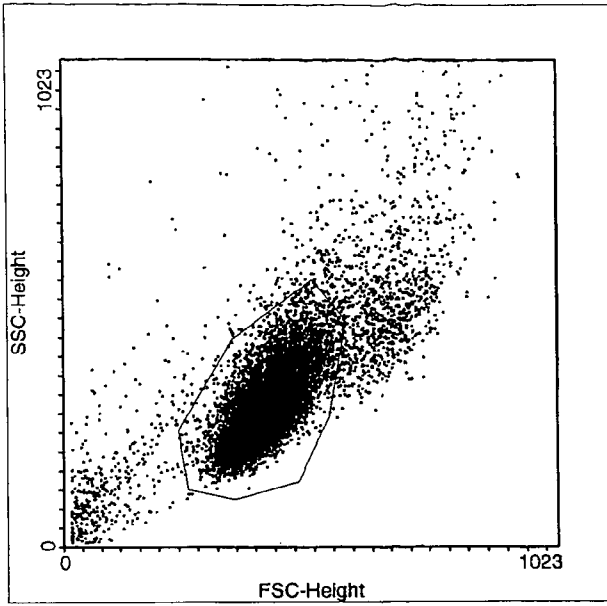
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:51:48 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI 0Gy 6h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 17411 87.06%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.2	178.1	2688	13.44	15.44
2 UR	12.2	174.6	6202	31.01	35.62
3 LL	1.0	2.5	1	0.01	0.01
4 LR	130.4	1.1	8520	42.60	48.93

51.06 → 69.8

AID = 1.04



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:52:08 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/DiI 9Gy 6h after  
 Date: 22-Apr-2 Parameters: /  
 Total Events 20000 Gated Events 16945 84.72%  
 System: Log Parameter Means: Geometric  
 Quad Stats

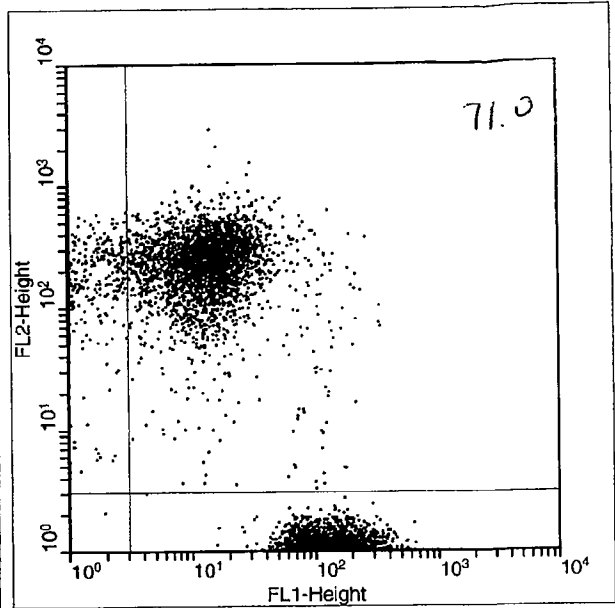
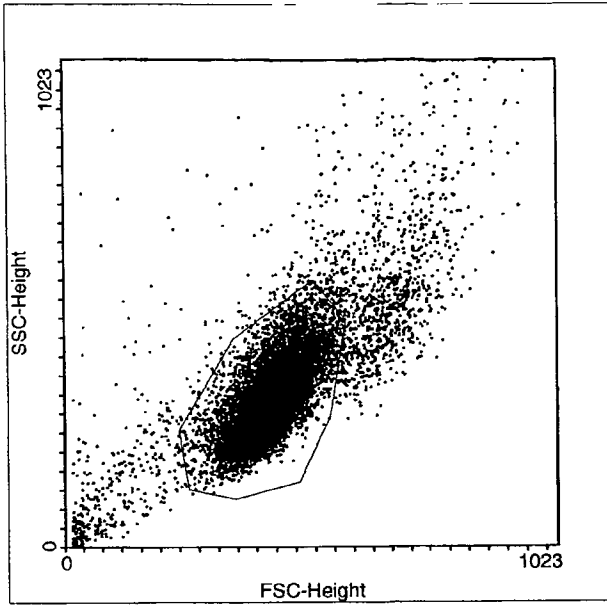
FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.2	174.7	3537	17.69	20.87
2 UR	10.5	175.3	5978	29.89	35.28
3 LL	0.0	0.0	0	0.00	0.00
4 LR	127.1	1.1	7430	37.15	43.85

} 56.15 → 62.8

AID = 1.28





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:52:29 2002

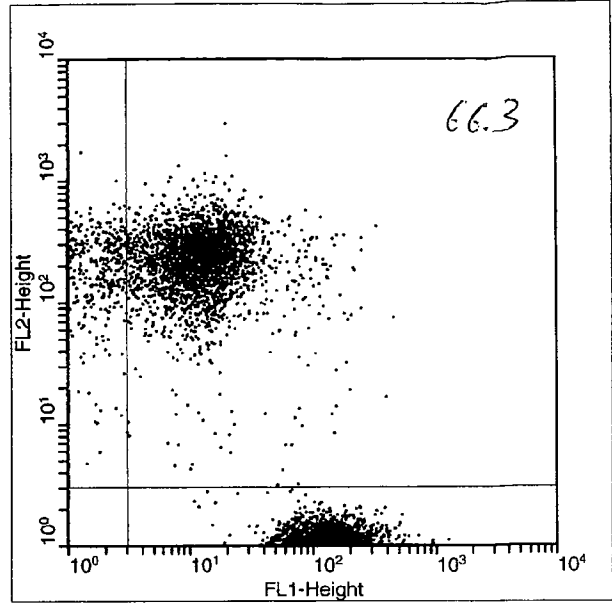
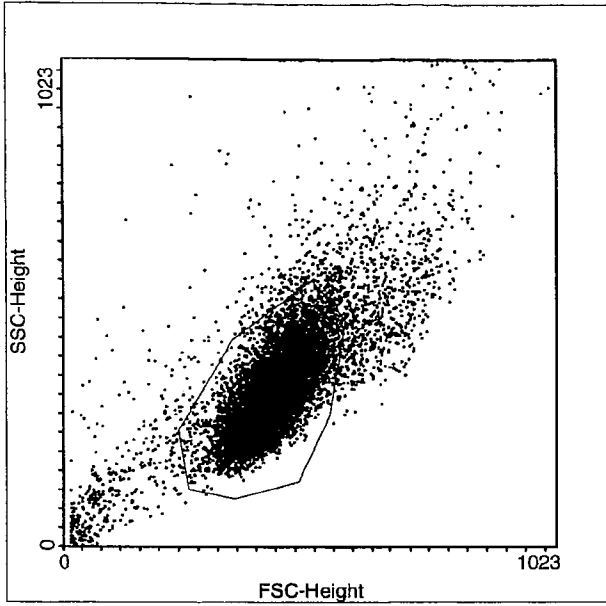
Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI\* 9Gy 6h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 17077 85.39%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	216.7	2266	11.33	13.27
2 UR	14.5	210.4	5534	27.67	32.41
3 LL	1.9	2.1	1	0.01	0.01
4 LR	137.4	1.1	9276	46.38	54.32

} 45.68 → 71.0

A/D = 0.84



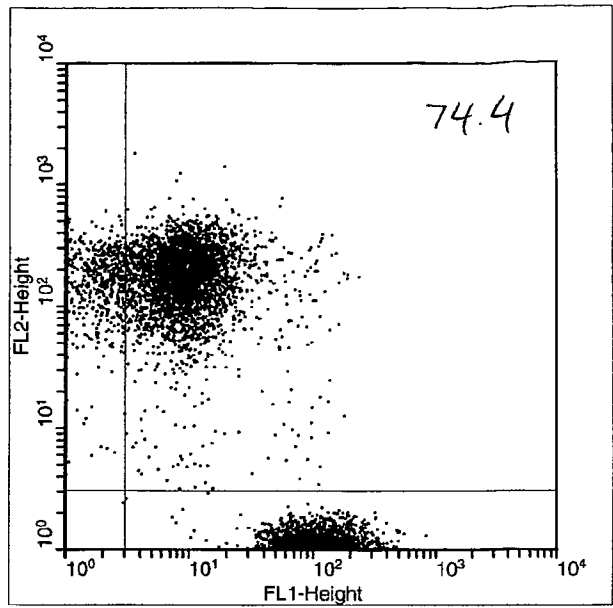
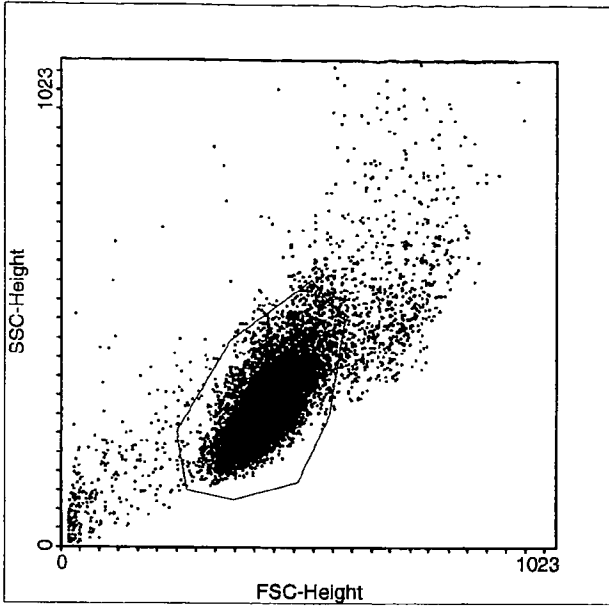
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:53:02 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample CA\*/DiI\* 9Gy 6h after  
 Date: 22-Apr-2 Parameters: 1  
 Total Events 20000 Gated Events 16987 84.93%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.1	213.1	2889	14.45	17.01
2 UR	12.4	211.3	5675	28.38	33.41
3 LL	0.0	0.0	0	0.00	0.00
4 LR	126.0	1.1	8423	42.12	49.58

} 50.42 → 66.3

A/D = 1.02



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:53:27 2002

Gates: R1

Project: Experiment:

File: 042202 Sample: CA/DiI 0Gy 8h after

Date: 22-Apr-2 Parameters: 7

Total Events 20000 Gated Events 17696 88.48%

System: Log Parameter Means: Geometric

Quad Stats

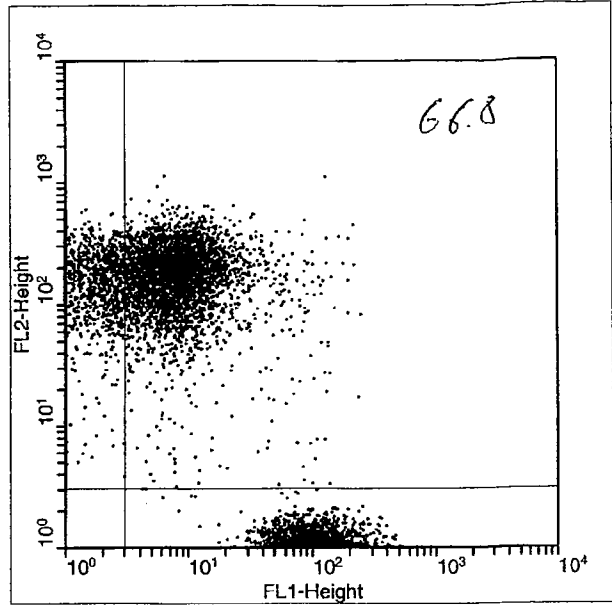
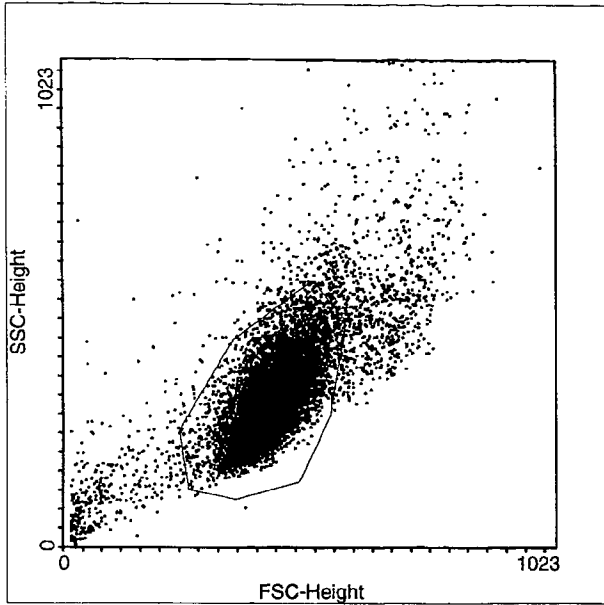
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.3	167.1	2270	11.35	12.83
2 UR	9.7	163.0	6581	32.91	37.19
3 LL	2.9	2.4	1	0.01	0.01
4 LR	110.4	1.1	8844	44.22	49.98

} 50.02 → 74.4

AID = 1.0



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:53:58 2002

Gates: R1

Project: Experiment:

File: 042202 Sample: CA\*/DiI 9Gy 8h after

Date: 22-Apr-2 Parameters: 7

Total Events 20000 Gated Events 17197 85.99%

System: Log Parameter Means: Geometric

Quad Stats

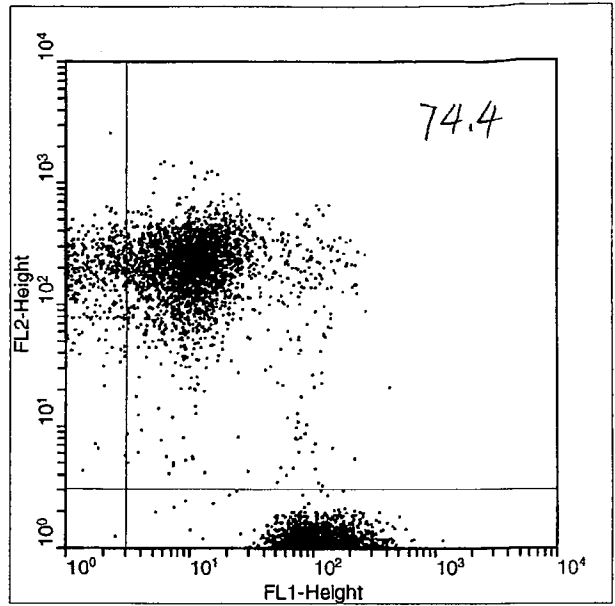
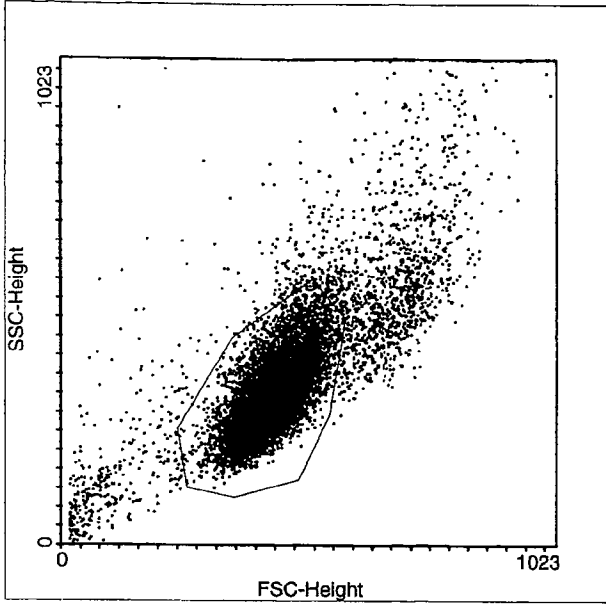
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.3	159.3	3322	16.61	19.32
2 UR	8.7	159.7	6680	33.40	38.84
3 LL	1.0	3.0	1	0.01	0.01
4 LR	102.9	1.1	7194	35.97	41.83

58.16 → 66.8

A/D = 1.39



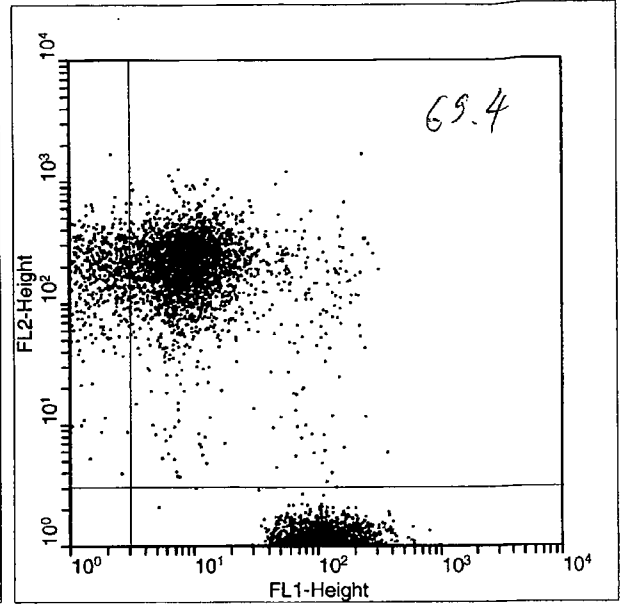
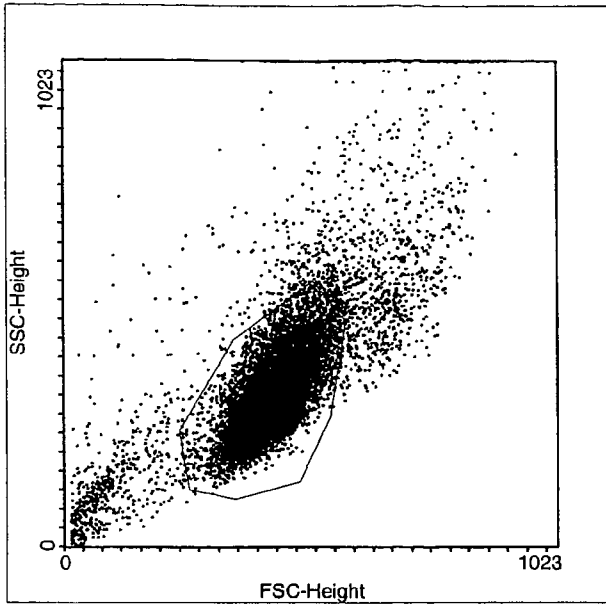
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:54:24 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA/DiI\* 9Gy 8h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16566 82.83%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.2	203.3	1968	9.84	11.88
2 UR	11.8	191.2	5710	28.55	34.47
3 LL	2.5	1.3	1	0.01	0.01
4 LR	119.3	1.1	8887	44.44	53.65

} 46.35 → 74.4

A/D = 0.86



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Apr 22 11:55:04 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042202 Sample: CA\*/Dii\* 9Gy 8h after  
 Date: 22-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16714 83.57%  
 System: Log Parameter Means: Geometric

Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 124,124

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.2	200.0	2656	13.28	15.89
2 UR	10.0	190.9	6020	30.10	36.02
3 LL	0.0	0.0	0	0.00	0.00
4 LR	111.4	1.1	8038	40.19	48.09

} 51.91 → 69.4

$A/D = 1.08$

A/D I. STAINING BEFORE IRRADIATION

SAMPLES \ TIME, h	0	4	6	8	COMMENTS
CA/D:I (0 Gy)	0.66	1.09	1.12	1.07	NO CHANGE
CA*/D:I (9 Gy)	0.86	1.16	1.17	1.22	↑
CA/D:I* (9 Gy)	0.74	1.12	1.06	1.03	↓
CA*/D:I* (9 Gy)	0.98	1.16	1.17	1.15	NO CHANGE



II. STAINING AFTER IRRADIATION

SAMPLES \ TIME, h	0	4	6	8	COMMENTS
CA/D:I (0 Gy)	0.62	1.04	1.04	1.0	NO CHANGE
CA*/D:I (9 Gy)	0.63	1.24	1.28	1.39	↑
CA/D:I* (9 Gy)	0.93	0.85	0.84	0.86	NO CHANGE
CA*/D:I* (9 Gy)	0.88	1.03	1.02	1.08	NO CHANGE



APRIL 23, 2002

- ⊕ CELL-TO-CELL COMMUNICATION  
(50/50 - STAINING)
- ⊕ IRRADIATION (96g) OF 50% CELL POPULATION
- ⊕ STAINING BEFORE IRRADIATION

WBs - (33p)

REPLICATE EXPERIMENT  
(TO EXPERIMENT OF APR. 16)

IRRADIATION TIME: 9:20

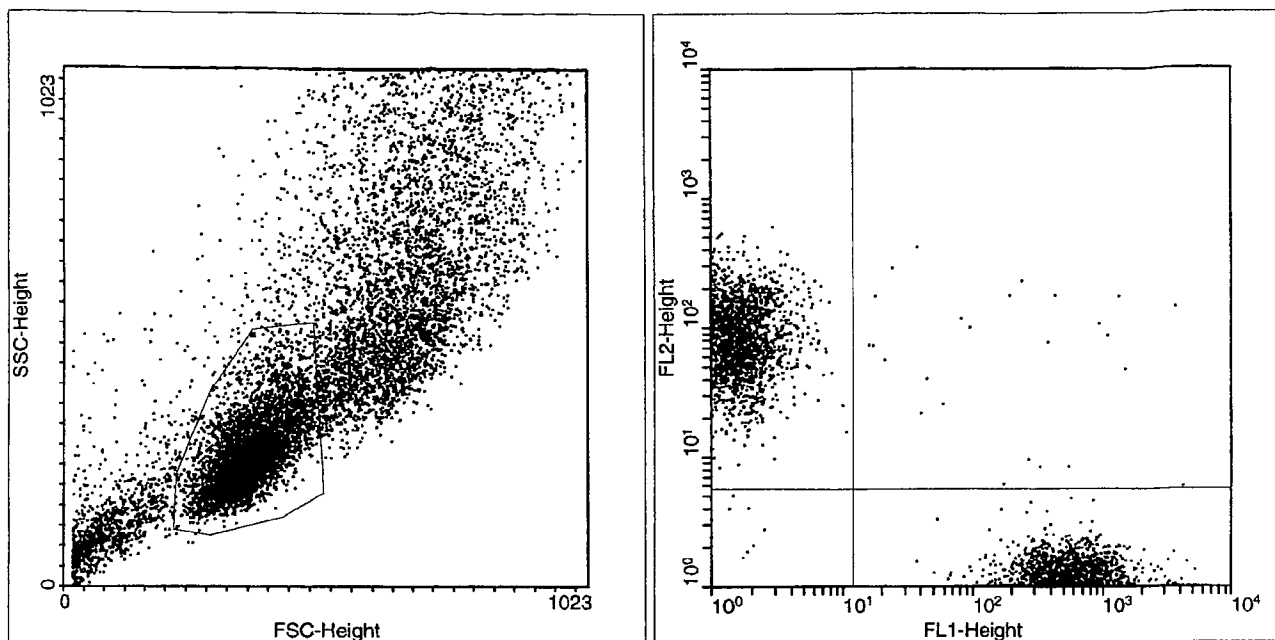
PLATING TIME: 10:10

HARVESTING TIME: 14:10 - (4h) - OK

16:10 - (1h) - OK

18:10 - (8h) - OK





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:12:18 2002

Gates: R1

Project: Experiment:

File: 042402 Sample: CA/DiI 0Gy 0h

Date: 24-Apr-2 Parameters: 7

Total Events 20000 Gated Events 7942 39.71%

System: Log Parameter Means: Geometric

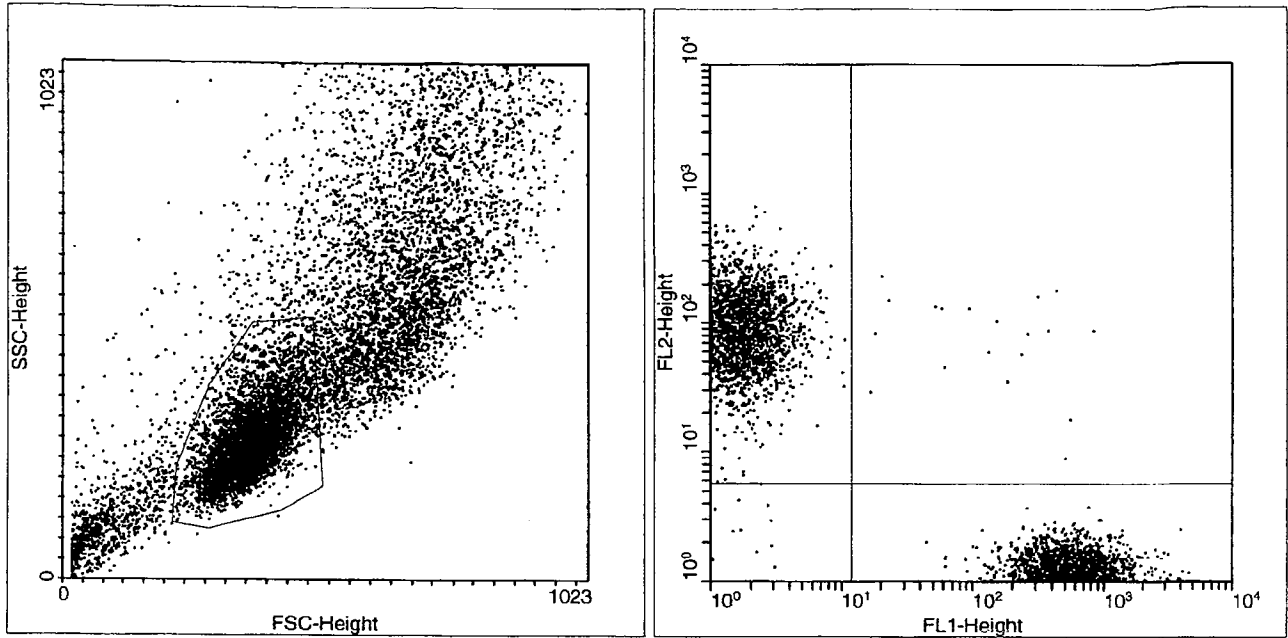
Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 276,192

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	86.9	3753	18.77	47.26
2 UR	132.6	54.5	51	0.26	0.64
3 LL	1.6	3.0	17	0.08	0.21
4 LR	618.6	1.2	4121	20.61	51.89

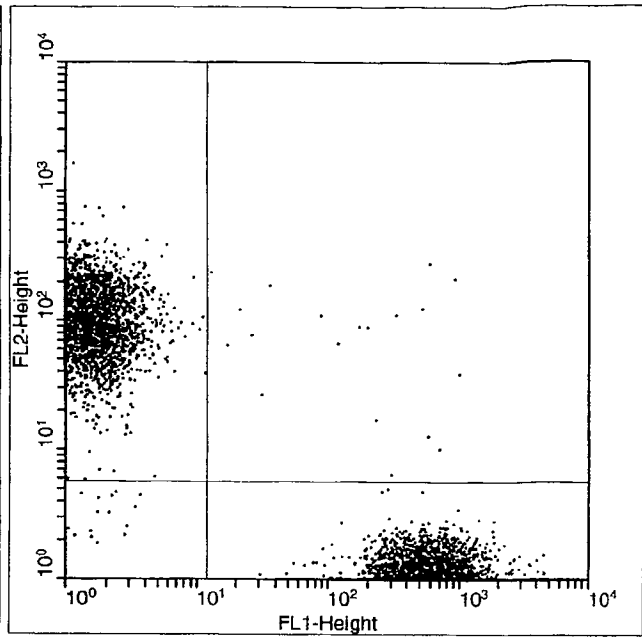
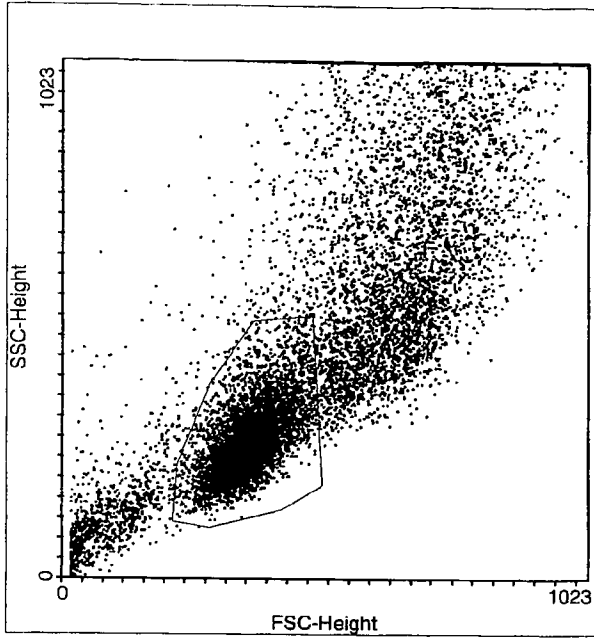
$$A/D = 0.91$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:14:11 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042402 Sample: CA\*/DiI 0Gy 0h  
 Date: 24-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 8428 42.14%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 276,192

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.8	87.2	3792	18.96	44.99
2 UR	122.3	68.3	39	0.20	0.46
3 LL	1.9	3.1	30	0.15	0.36
4 LR	588.2	1.2	4567	22.84	54.19

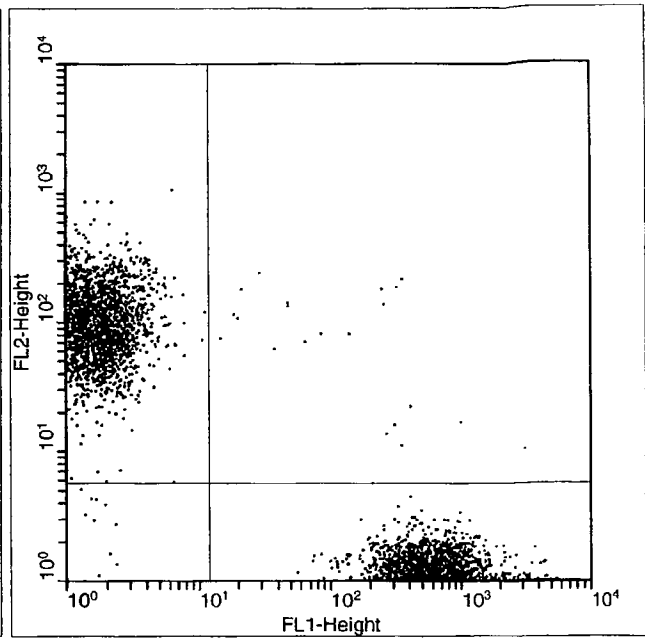
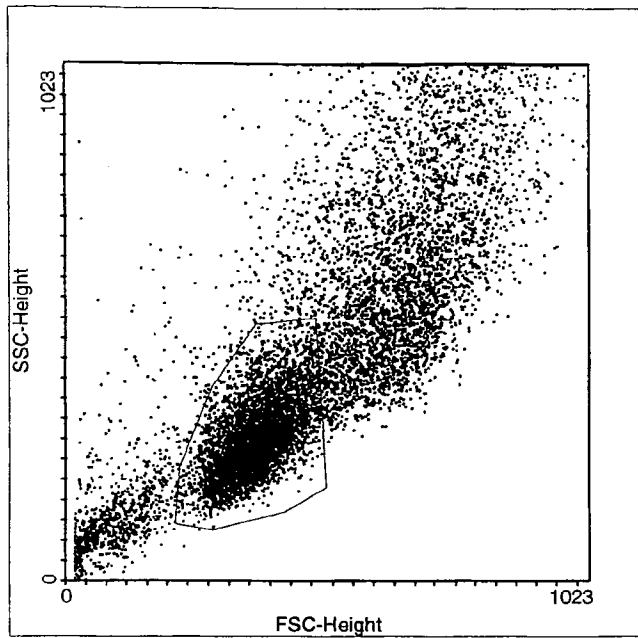
$$A/D = 0.83$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:14:46 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042402 Sample: CA/DiI\* 0Gy 0h  
 Date: 24-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 8326 41.63%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 276,192  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	96.3	4119	20.60	49.47
2 UR	120.8	68.3	45	0.23	0.54
3 LL	1.7	2.9	34	0.17	0.41
4 LR	658.7	1.2	4128	20.64	49.58

AID = 1.0



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:15:09 2002

Gates: R1

Project: Experiment:

File: 042402 Sample: CA\*/DiI\* 0Gy 0h

Date: 24-Apr-2 Parameters: 7

Total Events 20000 Gated Events 8510 42.55%

System: Log Parameter Means: Geometric

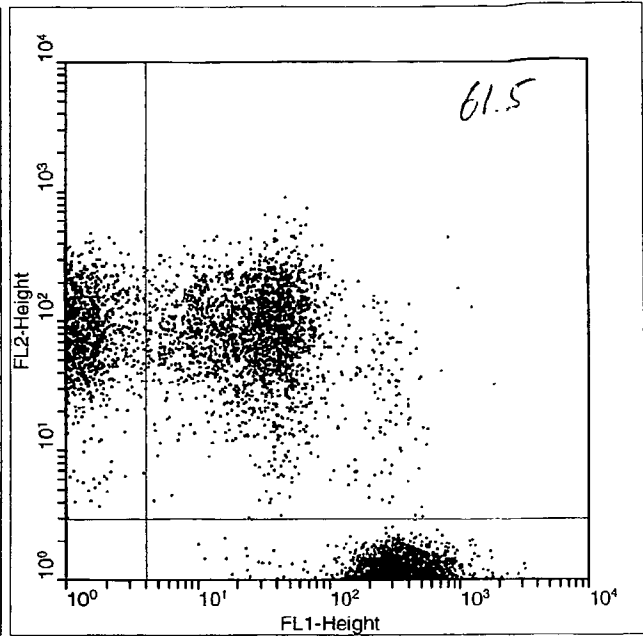
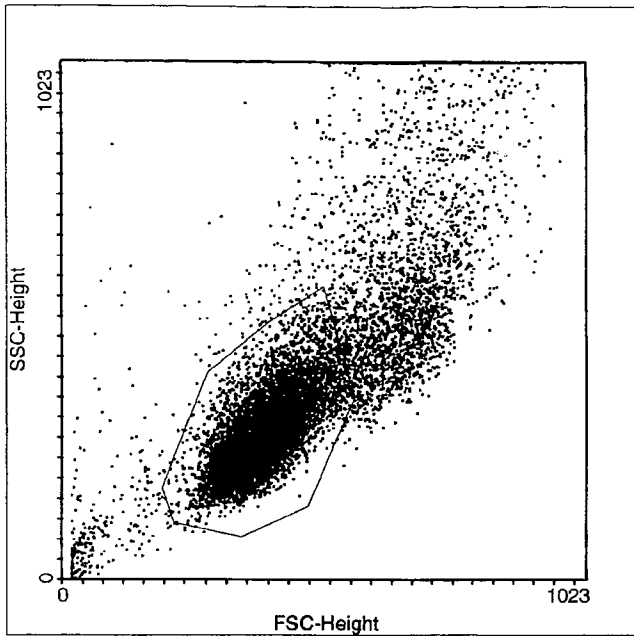
Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 276,192

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.7	98.0	3999	20.00	46.99
2 UR	112.1	61.3	49	0.25	0.58
3 LL	1.6	2.7	25	0.13	0.29
4 LR	649.8	1.2	4437	22.19	52.14

A/D = 0.90

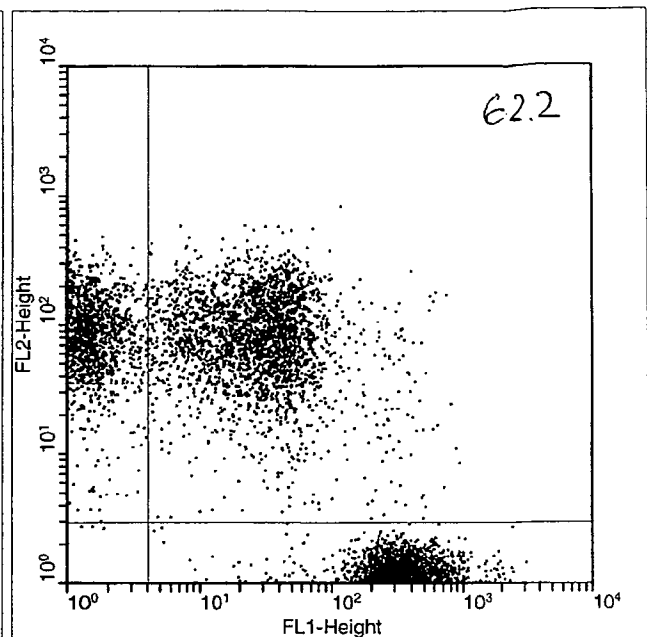
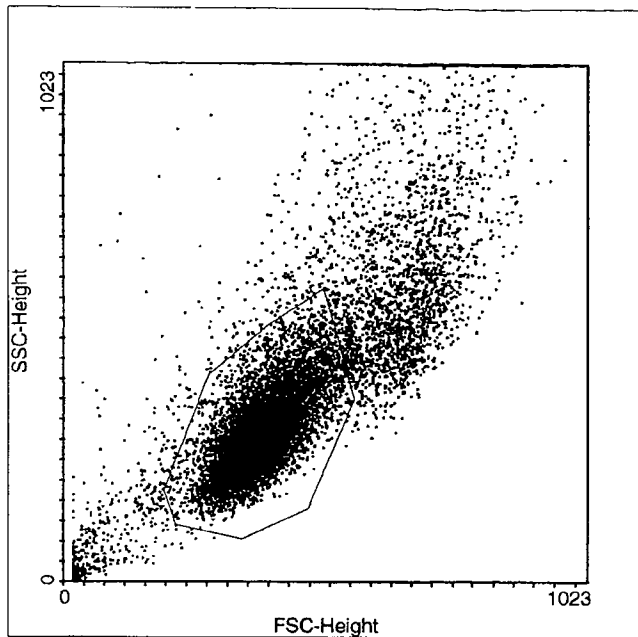


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:17:36 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042402 Sample: CA/DiI 0Gy 4h  
 Date: 24-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14328 71.64%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	88.7	2884	14.42	20.13
2 UR	29.5	76.6	4605	23.03	32.14
3 LL	1.4	1.9	4	0.02	0.03
4 LR	341.8	1.1	6835	34.18	47.70

} 52.27 → 61.5

A/D = 1.1



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:19:33 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042402 Sample: CA\*/DiI 9Gy 4h  
 Date: 24-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14513 72.57%  
 System: Log Parameter Means: Geometric

Quad Stats

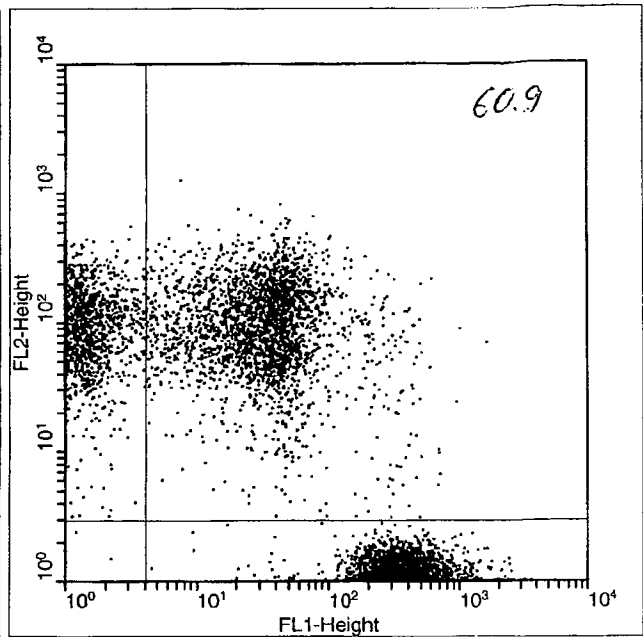
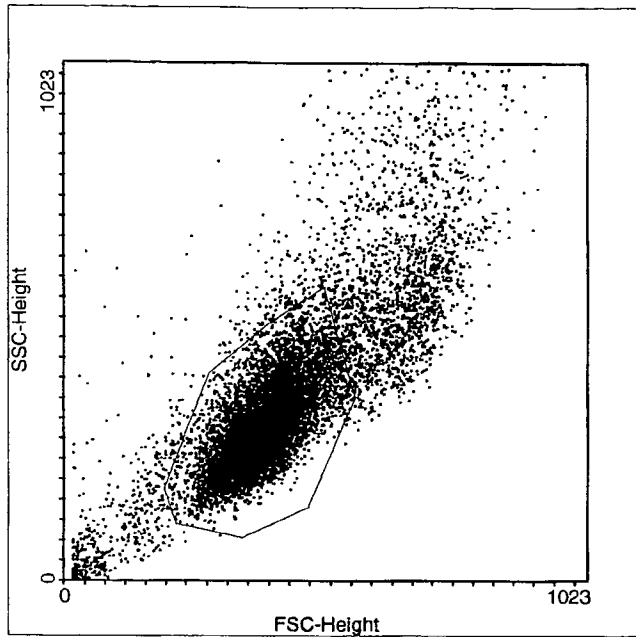
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.5	85.9	2922	14.61	20.13
2 UR	29.1	73.0	4801	24.01	33.08
3 LL	1.8	2.2	10	0.05	0.07
4 LR	344.4	1.1	6780	33.90	46.72

} 53.21 → 62.2

$$A/D = 1.14$$



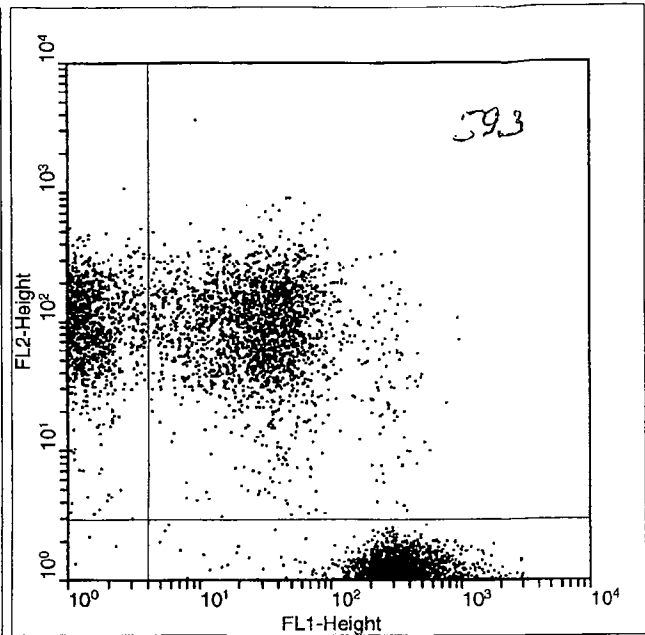
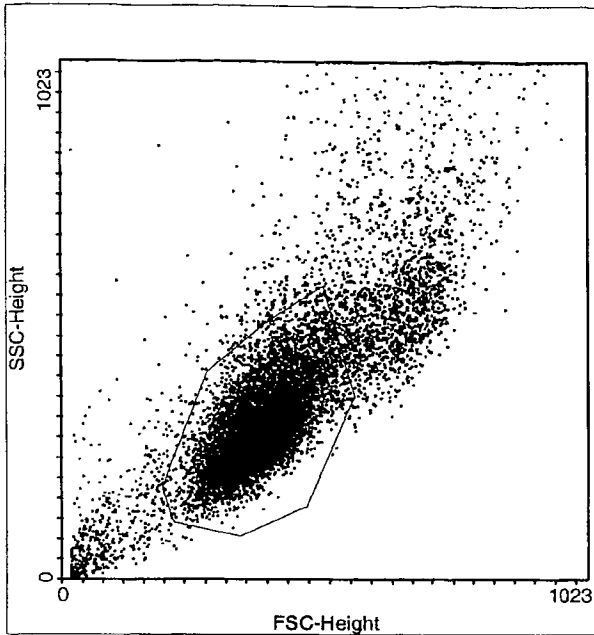
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:22:08 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042402 Sample: CA/DII\* 9Gy 4h  
 Date: 24-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14909 74.54%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	100.9	2948	14.74	19.77
2 UR	32.0	87.0	4598	22.99	30.84
3 LL	1.2	1.9	5	0.03	0.03
4 LR	365.3	1.1	7358	36.79	49.35

} 50.61 → 60.9

$$A/D = 1.23$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:22:30 2002

Gates: R1

Project: Experiment:

File: 042402 Sample: CA\*/DiI\* 9Gy 4h

Date: 24-Apr-2 Parameters: 7

Total Events 20000 Gated Events 14973 74.86%

System: Log Parameter Means: Geometric

Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

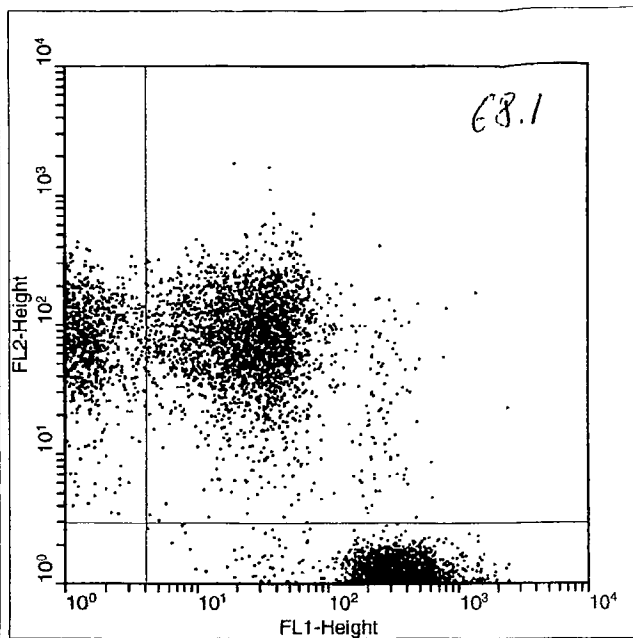
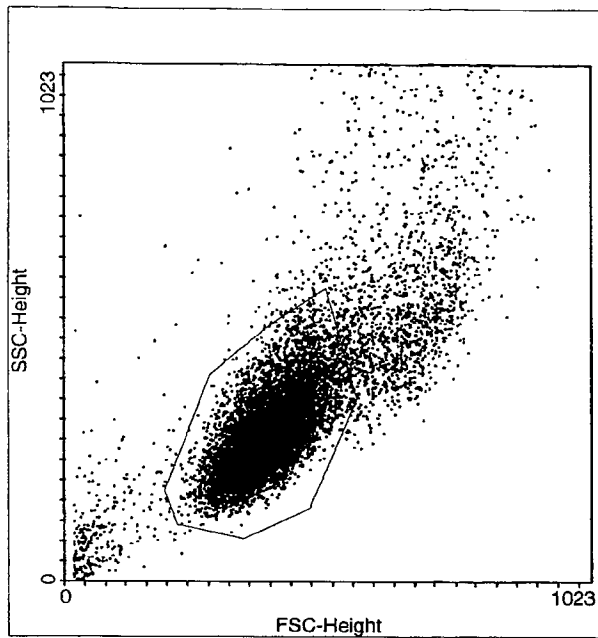
Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	98.8	3134	15.67	20.93
2 UR	29.8	85.7	4560	22.80	30.45
3 LL	1.4	2.0	11	0.06	0.07
4 LR	348.1	1.1	7268	36.34	48.54

} 51.38 → 59.3

A/D = 1.06





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:22:52 2002

Gates: R1

Project: Experiment:

File: 042402 Sample: CA/DIT 0Gy 6h

Date: 24-Apr-2 Parameters: 7

Total Events 20000 Gated Events 16122 80.61%

System: Log Parameter Means: Geometric

Quad Stats

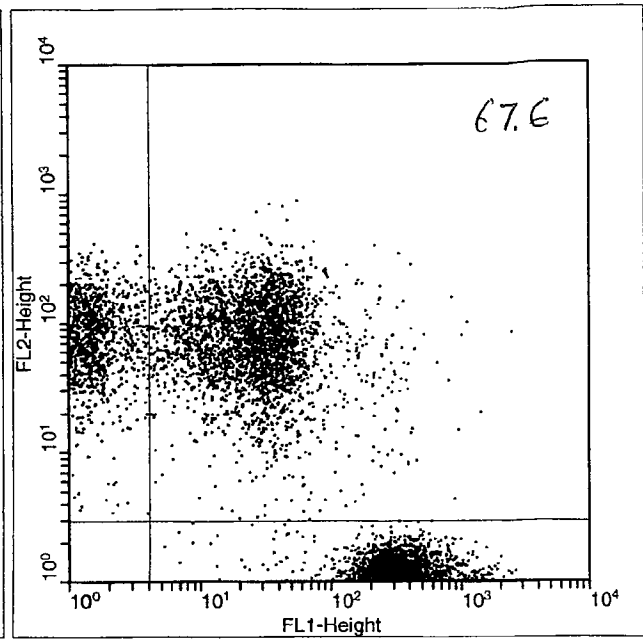
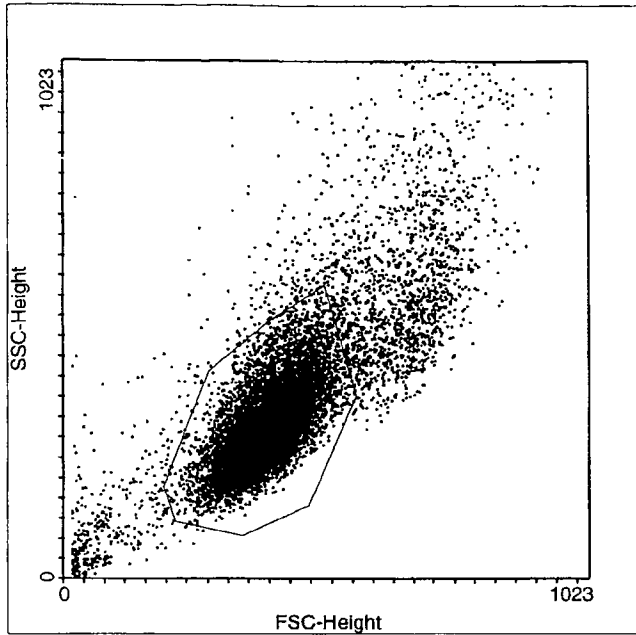
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	81.8	2616	13.08	16.23
2 UR	26.4	73.3	5595	27.98	34.70
3 LL	1.7	2.1	9	0.05	0.06
4 LR	329.7	1.1	7902	39.51	49.01

50.93 → 68.1

A/D = 1.04



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:23:11 2002

Gates: R1

Project: Experiment:

File: 042402 Sample: CA\*/DiI 9Gy 6h

Date: 24-Apr-2 Parameters: 7

Total Events 20000 Gated Events 15829 79.14%

System: Log Parameter Means: Geometric

Quad Stats

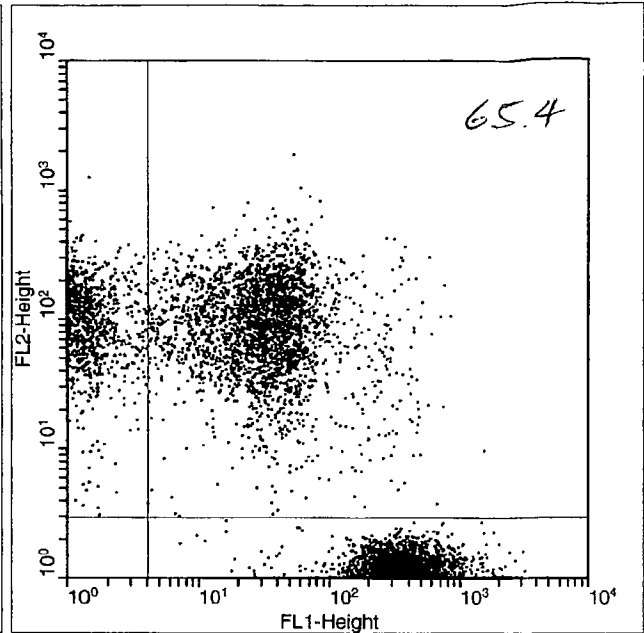
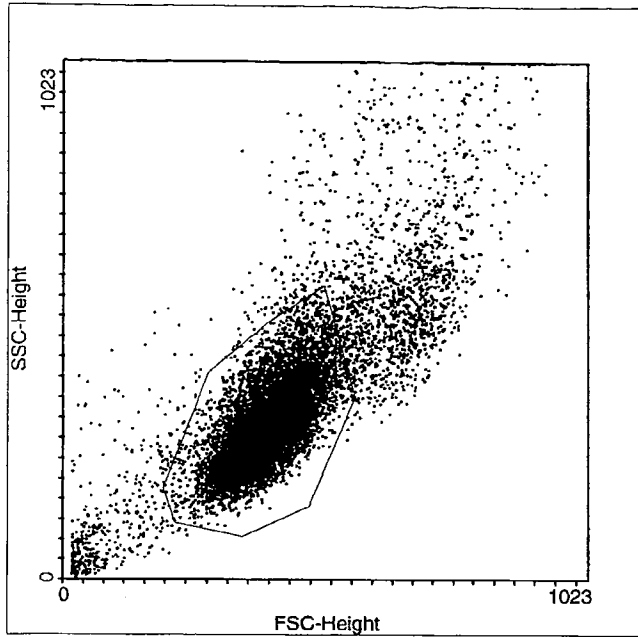
FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	84.1	2700	13.50	17.06
2 UR	26.0	71.0	5645	28.23	35.66
3 LL	1.5	2.6	4	0.02	0.03
4 LR	339.4	1.1	7480	37.40	47.26

} 52.72 → 67.6

$A/D = 1.12$



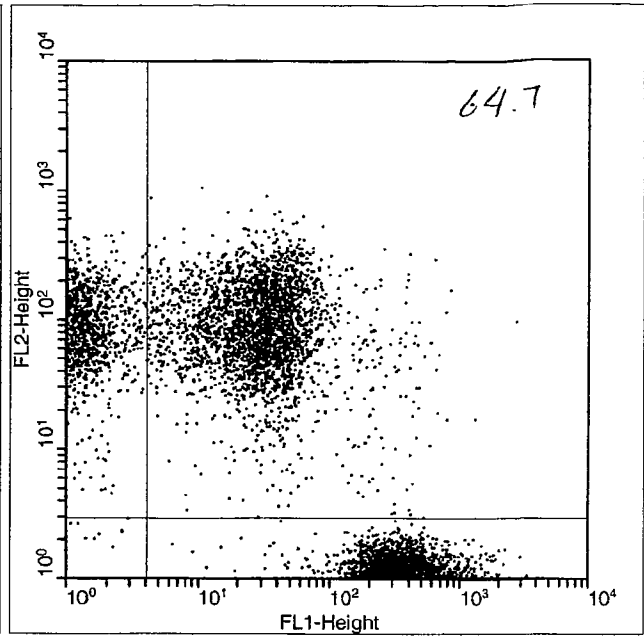
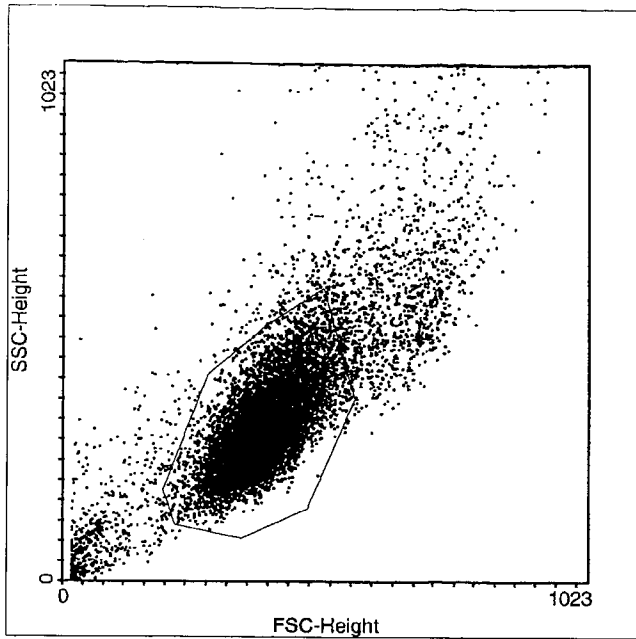
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:23:31 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042402 Sample: CA/DiI\* 9Gy 6h  
 Date: 24-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 15899 79.50%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.3	96.5	2661	13.31	16.74
2 UR	30.8	85.0	5039	25.20	31.69
3 LL	1.2	2.7	4	0.02	0.03
4 LR	362.5	1.1	8195	40.98	51.54

} 48.43 → 65.4

A/D = 0.94



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:23:53 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042402 Sample: CA\*/DiI\* 9Gy 6h  
 Date: 24-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16290 81.45%  
 System: Log Parameter Means: Geometric  
 Quad Stats

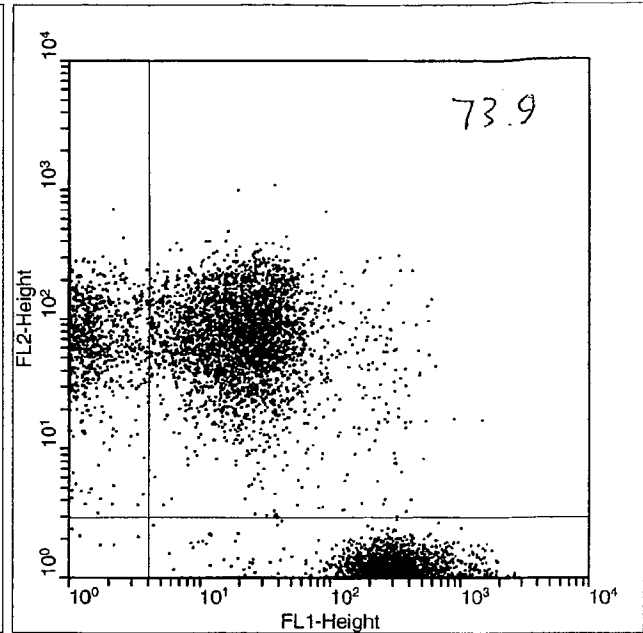
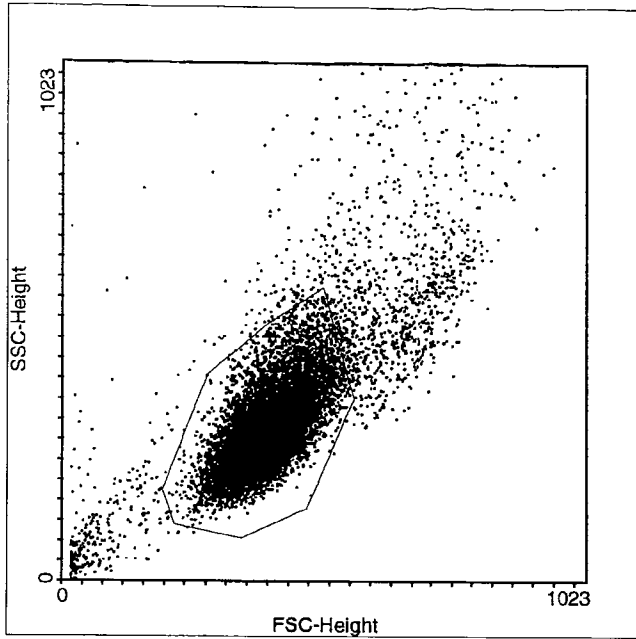
FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.3	96.6	2868	14.34	17.61
2 UR	29.3	82.1	5264	26.32	32.31
3 LL	1.6	2.2	12	0.06	0.07
4 LR	346.5	1.1	8146	40.73	50.01

} 49.92 → 64.7

A/D = 1.0



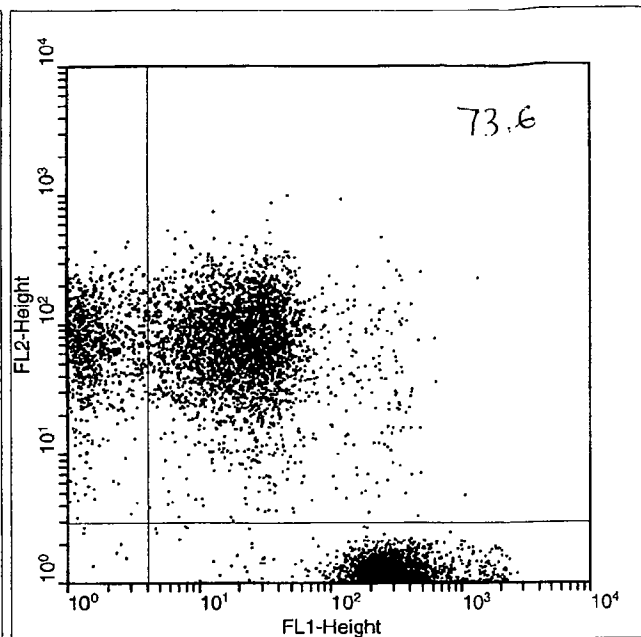
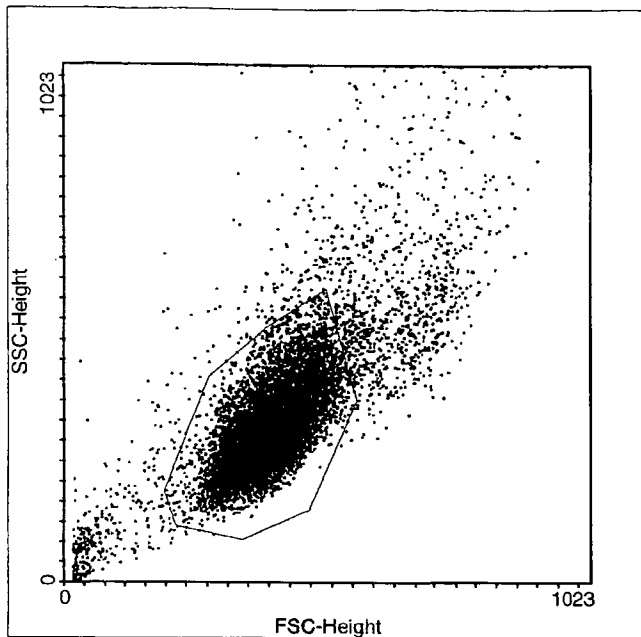
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:24:16 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042402 Sample: CA/DiI 0Gy 8h  
 Date: 24-Apr-2 Parameters:  
 Total Events 20000 Gated Events 17078 85.39%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	79.1	2268	11.34	13.28
2 UR	22.4	69.9	6435	32.18	37.68
3 LL	1.4	2.0	8	0.04	0.05
4 LR	313.9	1.1	8367	41.84	48.99

} 50.96 → 73.9

A/D = 1.04



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:24:40 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042402 Sample: CA\*/DiI 9Gy 8h  
 Date: 24-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 17028 85.14%  
 System: Log Parameter Means: Geometric

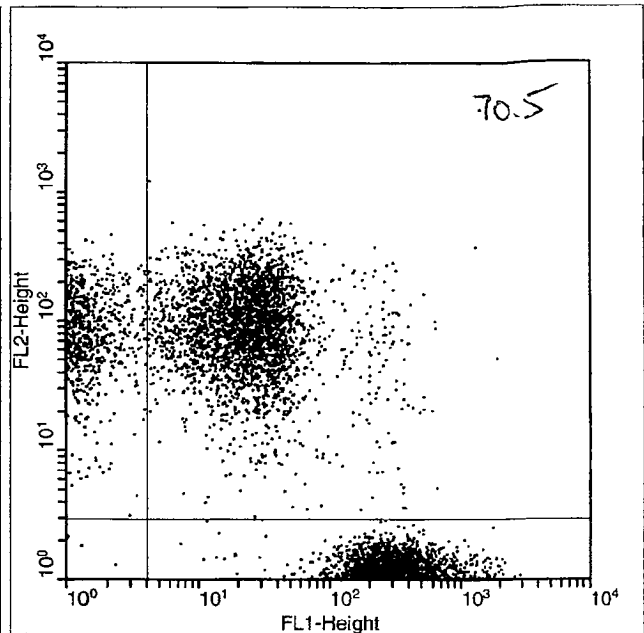
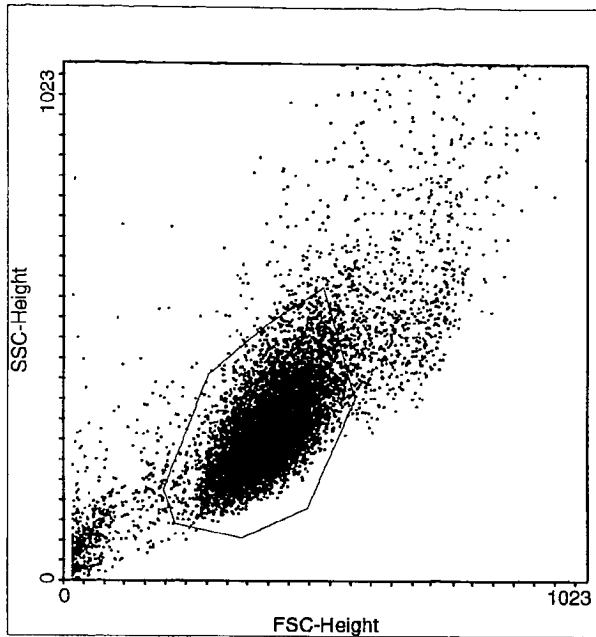
Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.4	76.3	2369	11.85	13.91
2 UR	22.0	69.5	6610	33.05	38.82
3 LL	2.0	1.8	10	0.05	0.06
4 LR	303.0	1.1	8039	40.20	47.21

52.73 → 73.6

$$A/D = 1.12$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:25:01 2002

Gates: R1

Project: Experiment:

File: 042402 Sample: CA/DiI\* 9Gy 8h

Date: 24-Apr-2 Parameters: 7

Total Events 20000 Gated Events 16658 83.29%

System: Log Parameter Means: Geometric

Quad Stats

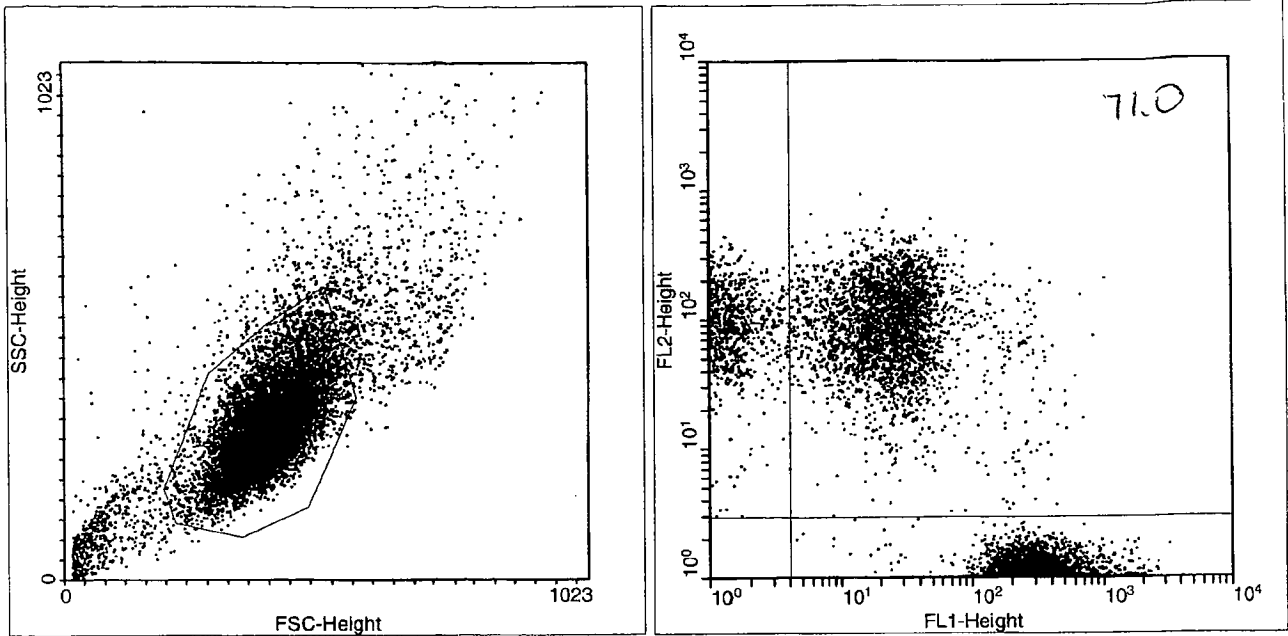
FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.3	94.9	2344	11.72	14.07
2 UR	24.4	85.2	5596	27.98	33.59
3 LL	1.6	1.6	5	0.03	0.03
4 LR	302.3	1.1	8713	43.57	52.31

} 47.66 → 70.5

A/D = 0.91



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Apr 24 11:25:22 2002  
 Gates: R1  
 Project: Experiment  
 File: 042402 Sample: CA\*/DiI\* 9Gy 8h  
 Date: 24-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16897 84.49%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 156,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.3	95.9	2376	11.88	14.06
2 UR	24.8	85.0	5817	29.09	34.43
3 LL	1.3	2.3	3	0.02	0.02
4 LR	303.8	1.1	8701	43.51	51.49

48.49 → 71.0

$$A/D = 0.94$$



REPLICATE EXPERIMENT (APR. 23)

①

	4h	6h	8h
CA / DI (0 Gy)	61.5	68.1	73.9
CA* / DI (96 Gy)	62.2	67.6	73.6
CA / DI* (96 Gy)	60.9	65.4	70.5
CA* / DI* (96 Gy)	59.3	64.7	71.0

② A/D RATIO

	0h	4h	6h	8h
CA / DI (0 Gy)	0.91	1.1	1.04	1.04
CA* / DI (96 Gy)	0.83	1.14	1.12	1.12
CA / DI* (96 Gy)	1.0	1.03	0.94	0.91
CA* / DI (96 Gy)	0.90	1.06	1.0	0.94

Street Address:

  
(e.g., 600 Mountain Ave, or Mountain Ave & South St, or just Mountain Ave)

City, State  
Zipcode:

  
Newark, NJ 07103  
(e.g., Murray Hill, NJ 07974, or Murray Hill, NJ, or 07974, or just NJ)

[Plan Route >](#)

[Clear](#)

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Preserving the Trust

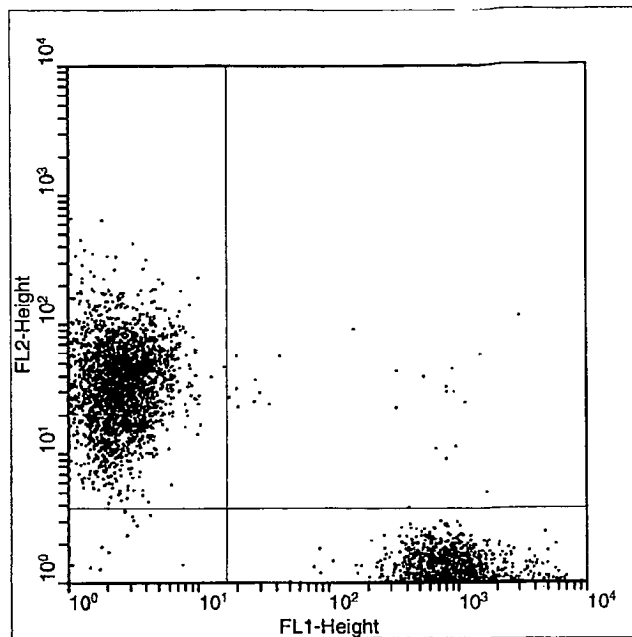
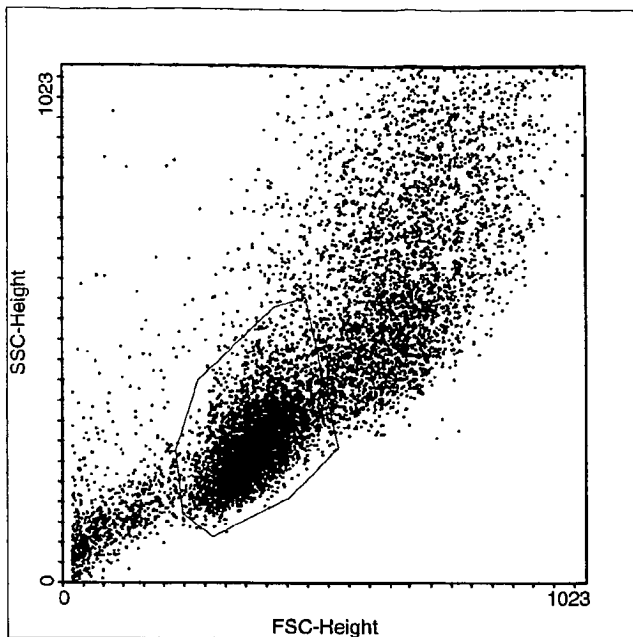
[español](#) - [site map](#) - [contact us](#) - [FAQs](#) - [search](#) - [keywords](#)  
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APRIL 25, 2002

- \* CELL-TO-CELL COMMUNICATION  
(50/50 - STAINING)
- \* IRRADIATION (96y) OF 50% CELL POPULATION
- \* STAINING AFTER IRRADIATION

WBs - 33A

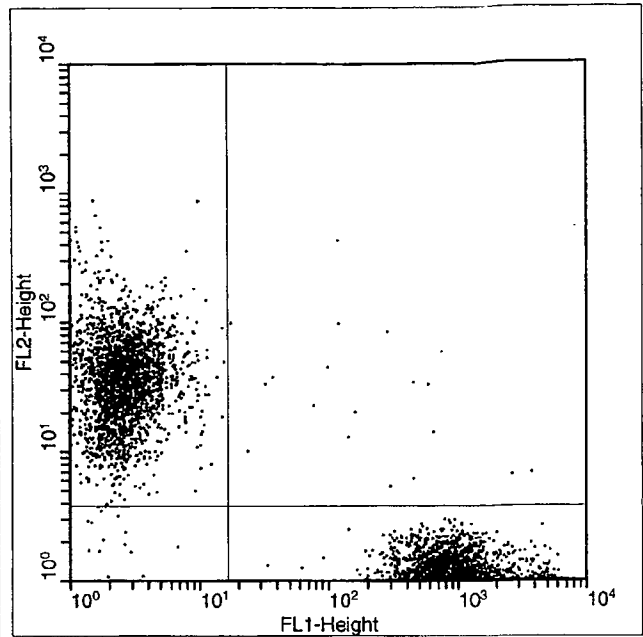
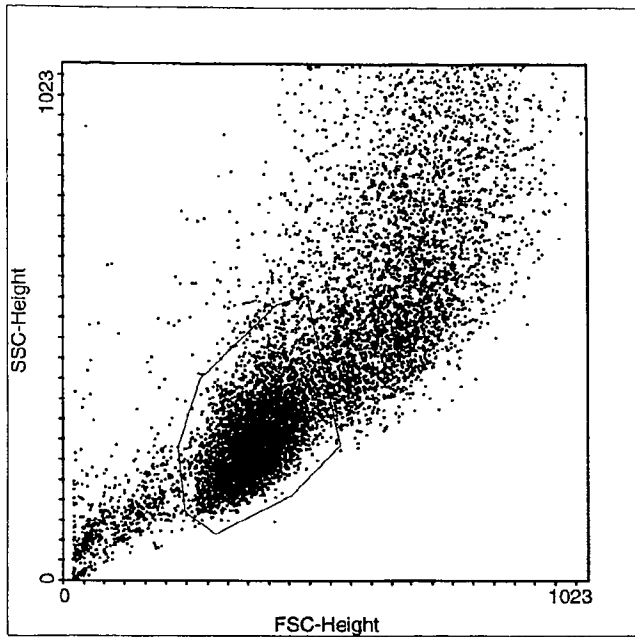
IRRADIATION TIME:	8:20	
PLATING TIME:	10:00	- OK
HARVESTING TIME:	14:00	- OK
	16:00	- OK
	18:00	- OK



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:31:47 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA/DiI 0Gy 0h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 9018 45.09%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 312,148  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.5	35.3	4795	23.98	53.17
2 UR	231.5	25.9	44	0.22	0.49
3 LL	2.5	2.5	25	0.13	0.28
4 LR	1205.9	1.1	4154	20.77	46.06

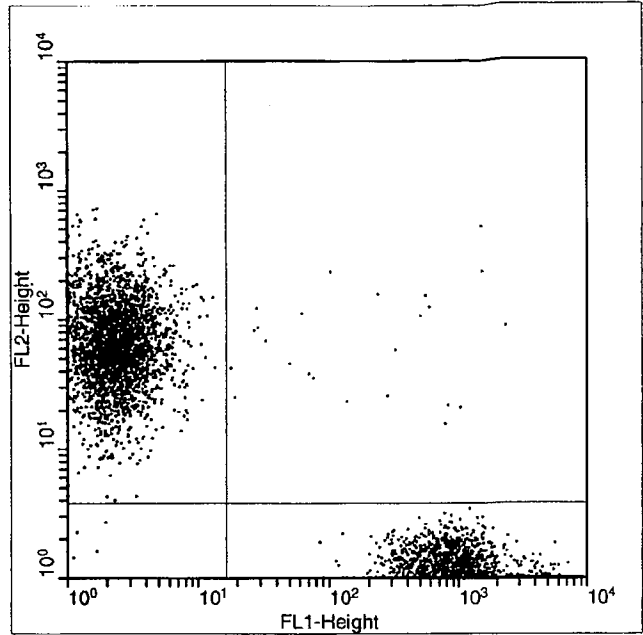
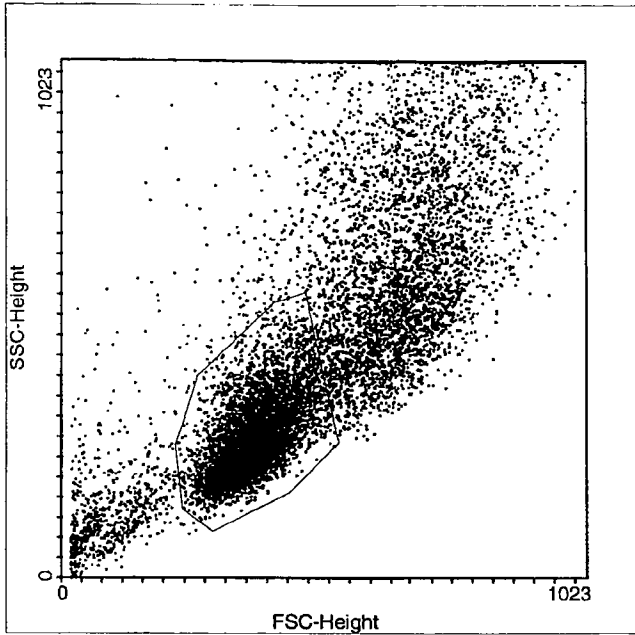
AID = 1.15



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:34:50 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA\*/DiI 9Gy 0h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 9550 47.75%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 312,148  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.6	36.3	4178	20.89	43.75
2 UR	185.8	25.1	37	0.19	0.39
3 LL	2.2	2.0	21	0.11	0.22
4 LR	1264.7	1.1	5314	26.57	55.64

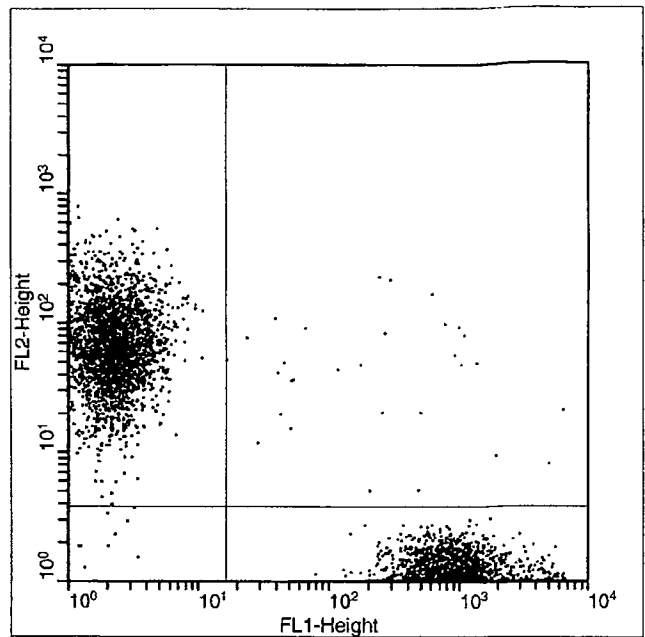
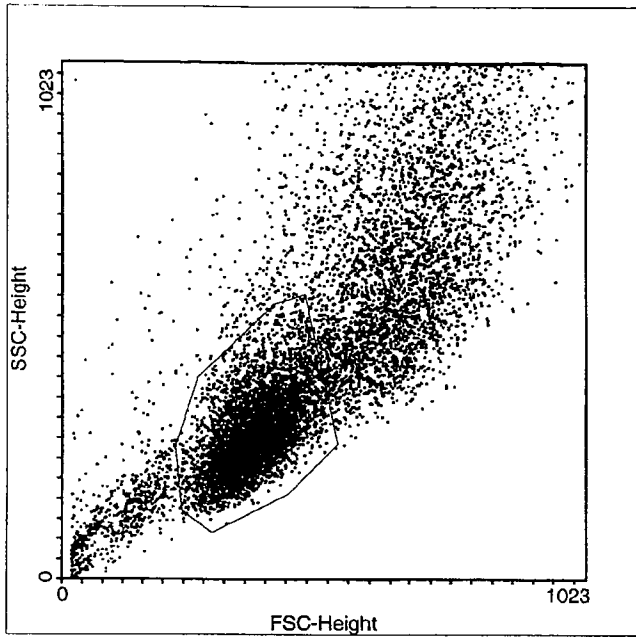
$$A/D = 0.79$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:35:12 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA/DiI\* 9Gy 0h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 9287 46.44%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 312,148  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.4	67.1	5388	26.94	58.02
2 UR	186.2	57.1	41	0.21	0.44
3 LL	1.4	2.2	7	0.04	0.08
4 LR	1167.3	1.2	3851	19.26	41.47

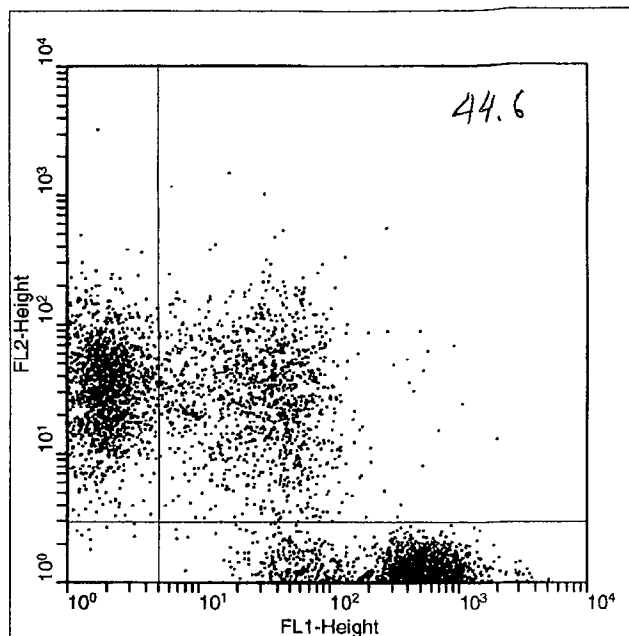
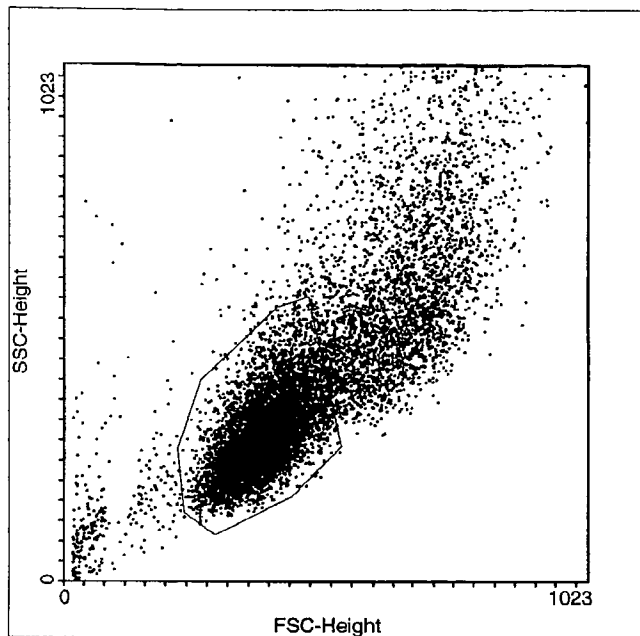
$$A/D = 1.4$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:35:36 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA\*/DiI\* 9Gy 0h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 9791 48.96%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 312,148  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.2	66.6	4790	23.95	48.92
2 UR	184.2	44.5	49	0.25	0.50
3 LL	1.8	2.2	18	0.09	0.18
4 LR	1278.8	1.1	4934	24.67	50.39

A/D = 0.97



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:38:03 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA/DiI 0Gy Ah  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 12650 63.25%  
 System: Log Parameter Means: Geometric

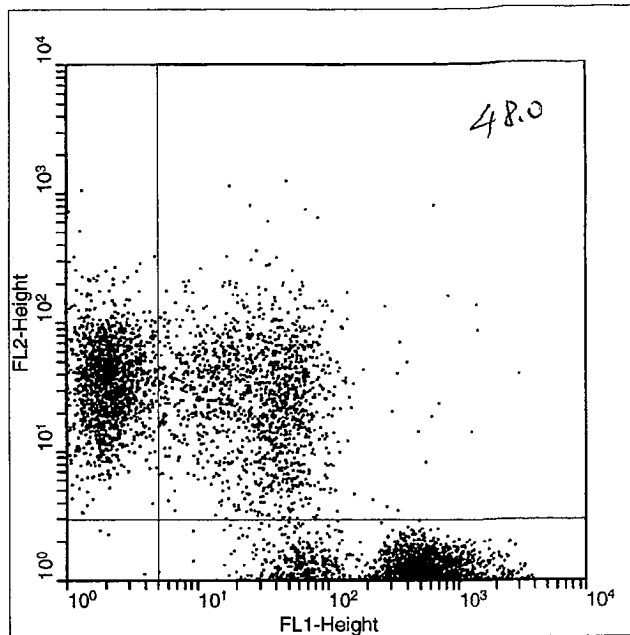
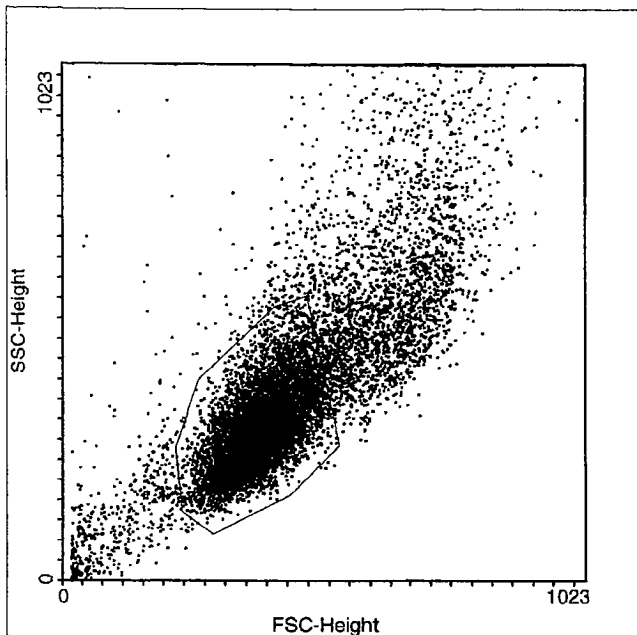
Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.0	34.4	3173	15.87	25.08
2 UR	30.8	27.5	2556	12.78	20.21
3 LL	1.9	2.3	12	0.06	0.09
4 LR	432.1	1.1	6909	34.54	54.62

45.29 → 44.6

$A/D = 0.83$





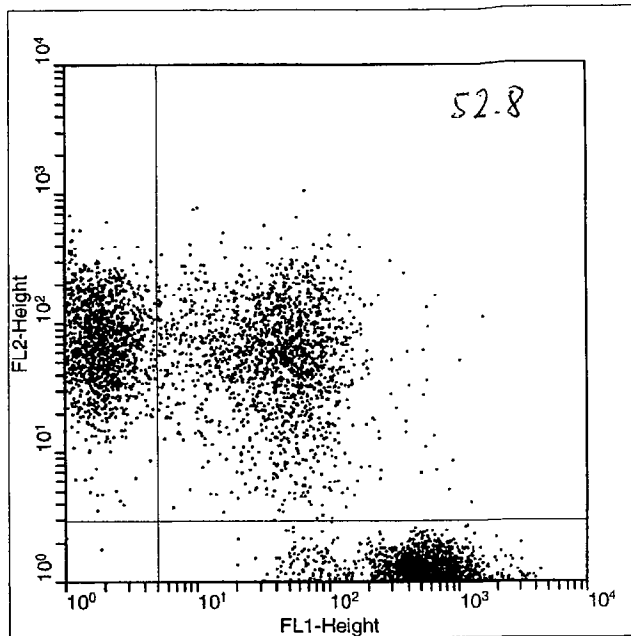
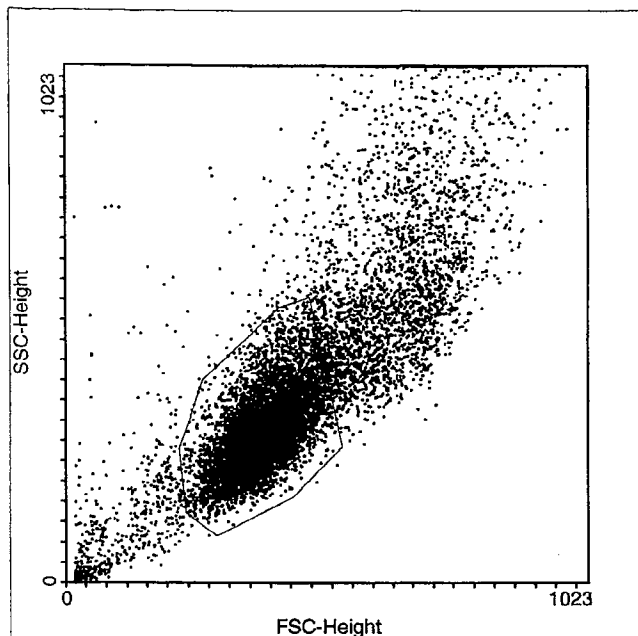
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:39:30 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA\*/DiT 9Gy 4h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13411 67.06%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.0	35.1	2984	14.92	22.25
2 UR	28.5	26.6	2750	13.75	20.51
3 LL	2.8	2.1	11	0.06	0.08
4 LR	466.2	1.1	7666	38.33	57.16

} 42.76 → 48.0

$AID = 0.75$



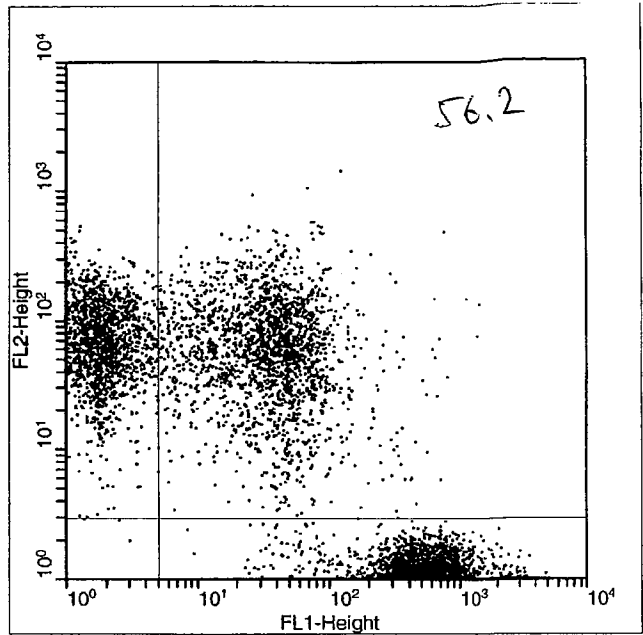
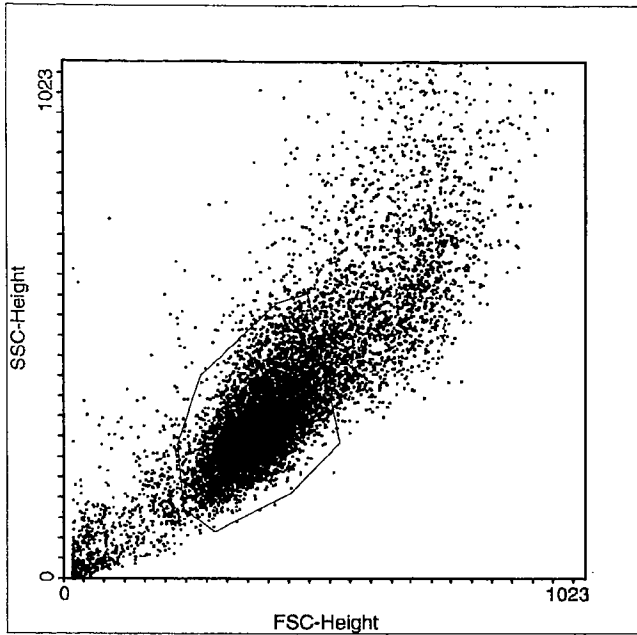
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:40:15 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA/DiI\* 9Gy 4h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13187 65.93%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.9	71.0	2975	14.88	22.56
2 UR	38.6	53.5	3324	16.62	25.21
3 LL	1.9	2.1	7	0.04	0.05
4 LR	555.7	1.1	6881	34.41	52.18

47.77 → 52.8

$A/D = 0.92$



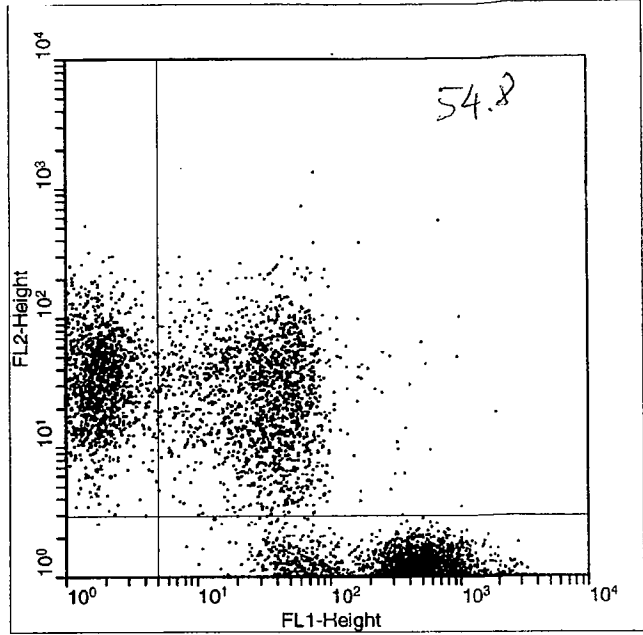
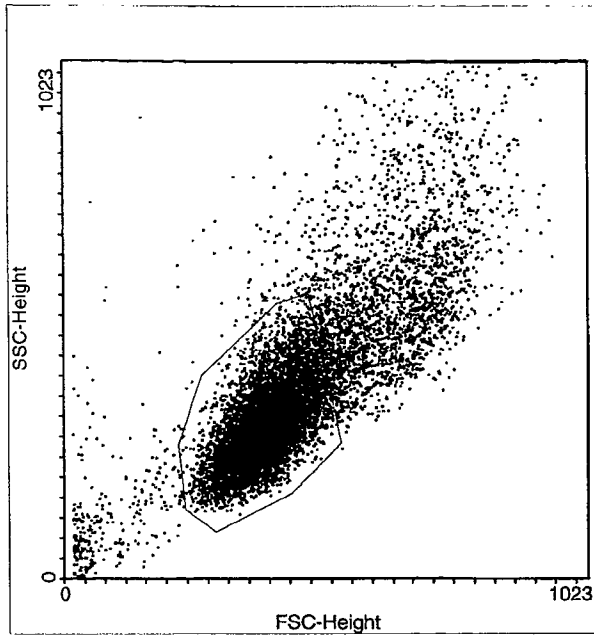
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:41:04 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA\*/DiI\* 9Gy 4h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 13489 67.44%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.8	69.5	2964	14.82	21.97
2 UR	34.1	55.8	3809	19.05	28.24
3 LL	1.8	2.5	7	0.04	0.05
4 LR	569.7	1.1	6709	33.55	49.74

50.21 → 56.2

A/D = 1.01



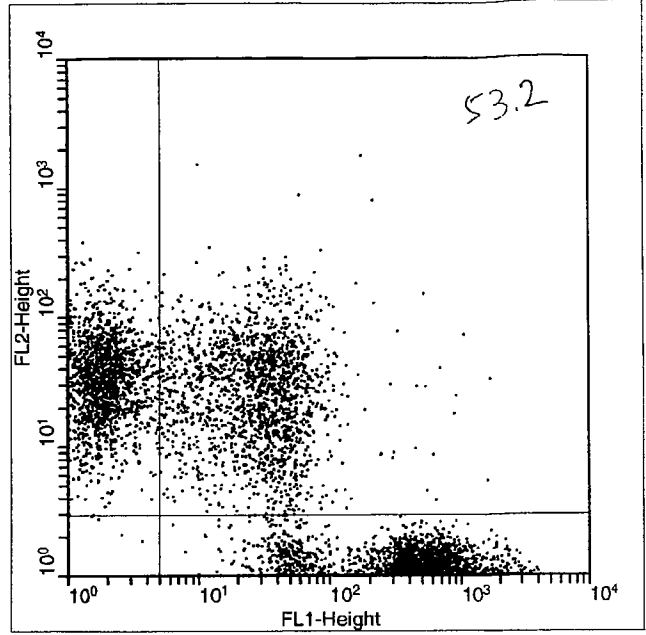
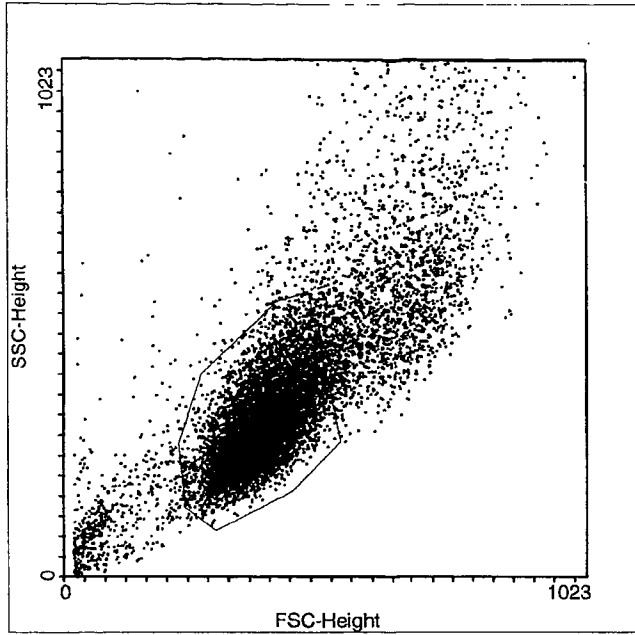
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:41:49 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA/DiI 0Gy 6h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14059 70.29%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.8	34.2	2686	13.43	19.11
2 UR	32.1	25.3	3256	16.28	23.16
3 LL	1.8	1.9	8	0.04	0.06
4 LR	410.0	1.1	8109	40.54	57.68

} 42.27 → 54.8

A(0) = 0.73



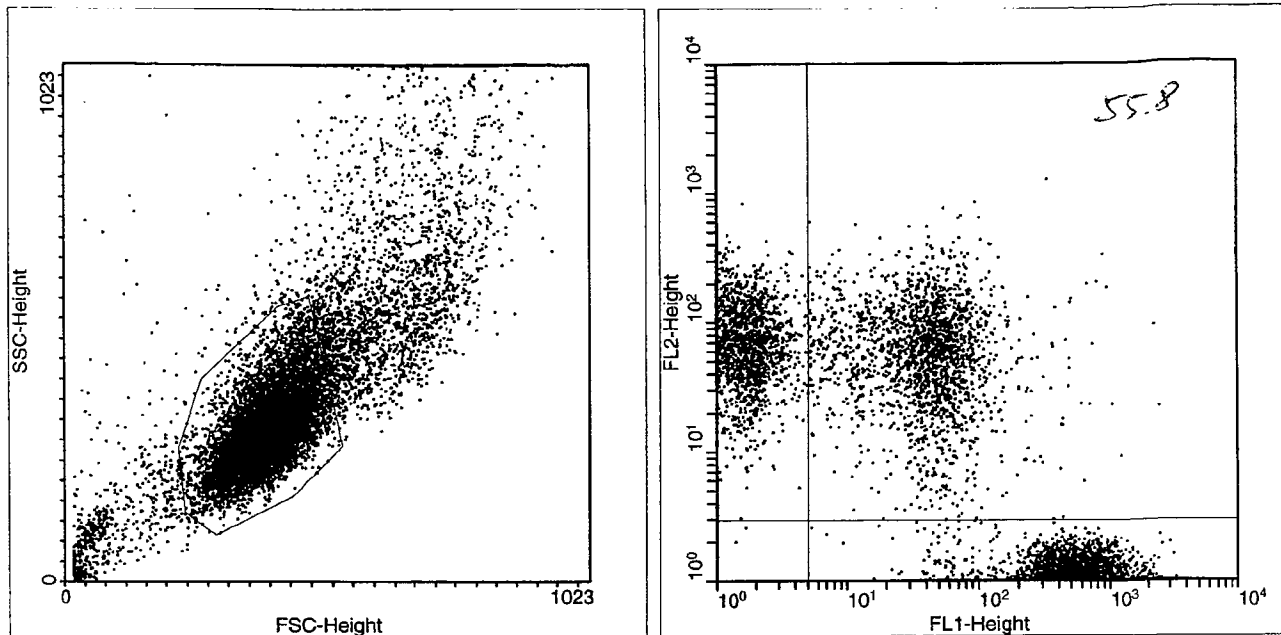
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:42:38 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA\*/DiI 9Gy 6h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14093 70.46%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.9	33.7	2916	14.58	20.69
2 UR	27.6	25.4	3316	16.58	23.53
3 LL	1.8	2.3	14	0.07	0.10
4 LR	449.7	1.1	7847	39.24	55.68

44.22 → 53.2

A/D = 0.79



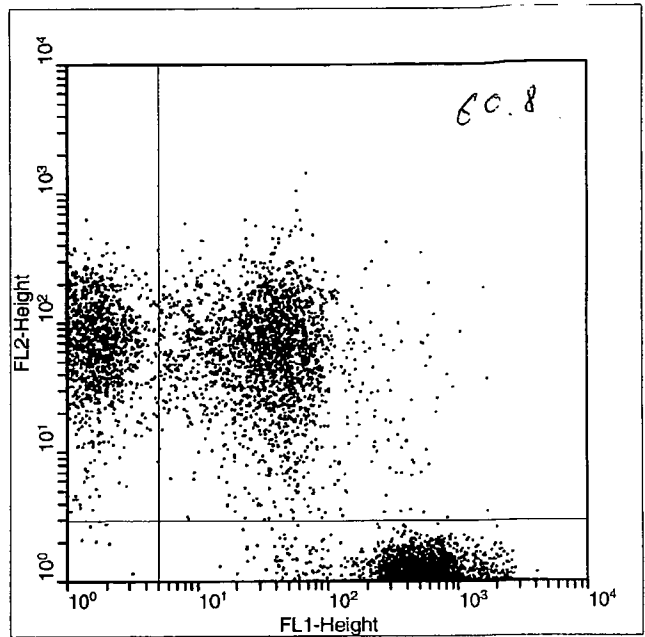
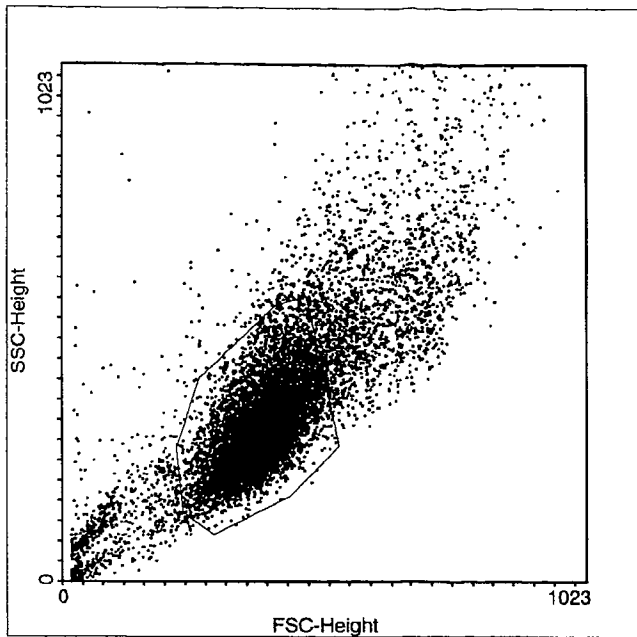
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:43:09 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA/DII\* 9Gy 6h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14154 70.77%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.7	70.9	3005	15.03	21.23
2 UR	38.1	54.0	3792	18.96	26.79
3 LL	1.4	2.1	5	0.03	0.04
4 LR	527.5	1.1	7352	36.76	51.94

48.02 → 55.8

$$A/D = 0.92$$



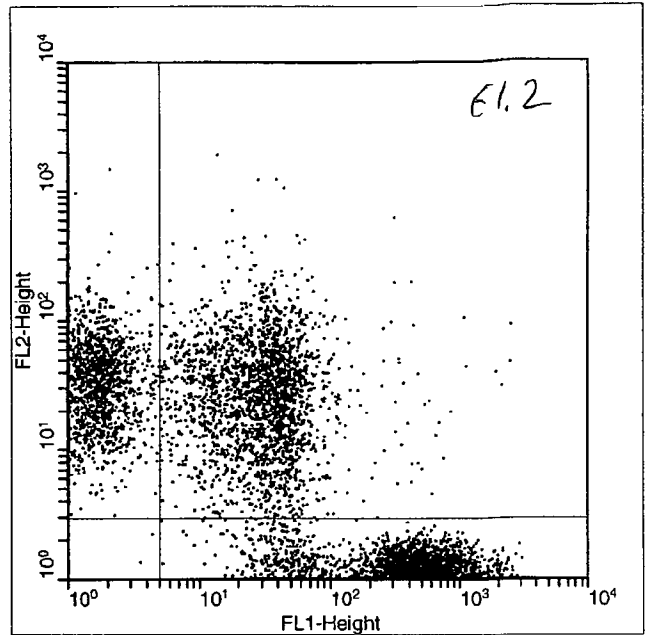
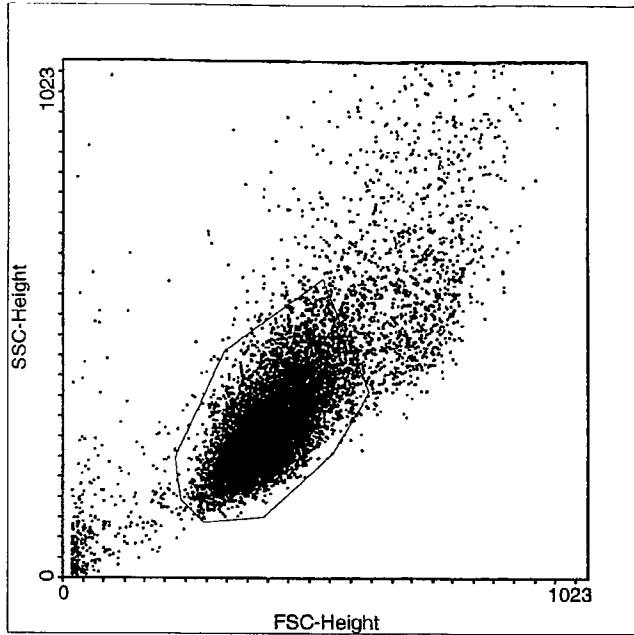
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:44:19 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA\*/DiI\* 9Gy 6h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 14423 72.11%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.7	69.9	2720	13.60	18.86
2 UR	34.9	54.7	4226	21.13	29.30
3 LL	1.9	2.0	10	0.05	0.07
4 LR	551.3	1.1	7467	37.34	51.77

} 48.16 → 60.8

A(D) = 0.93



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:47:23 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA/DII Ogy 8h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 16465 82.32%  
 System: Log Parameter Means: Geometric

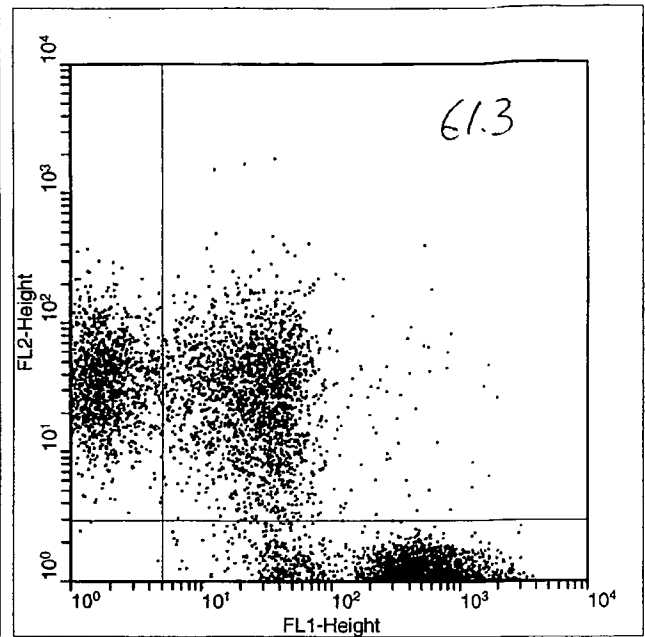
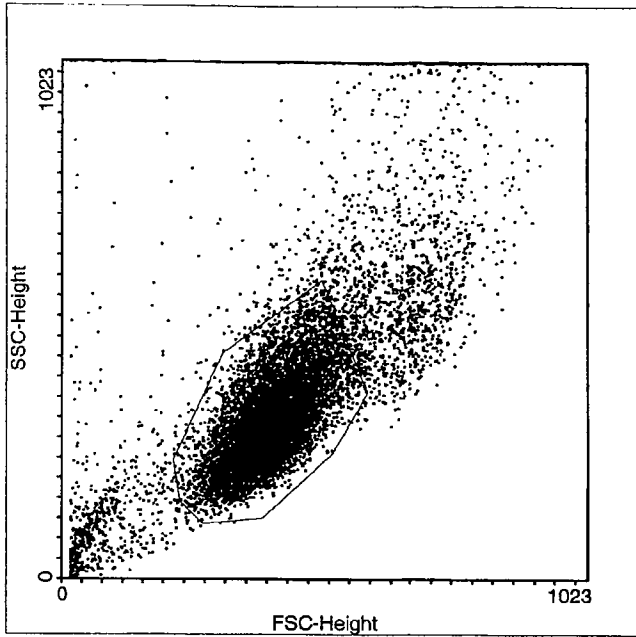
Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.7	33.7	2644	13.22	16.06
2 UR	28.4	26.1	4174	20.87	25.35
3 LL	2.3	2.2	10	0.05	0.06
4 LR	410.0	1.1	9637	48.19	58.53

} 41.41 → 61.2

$$A/D = 0.71$$





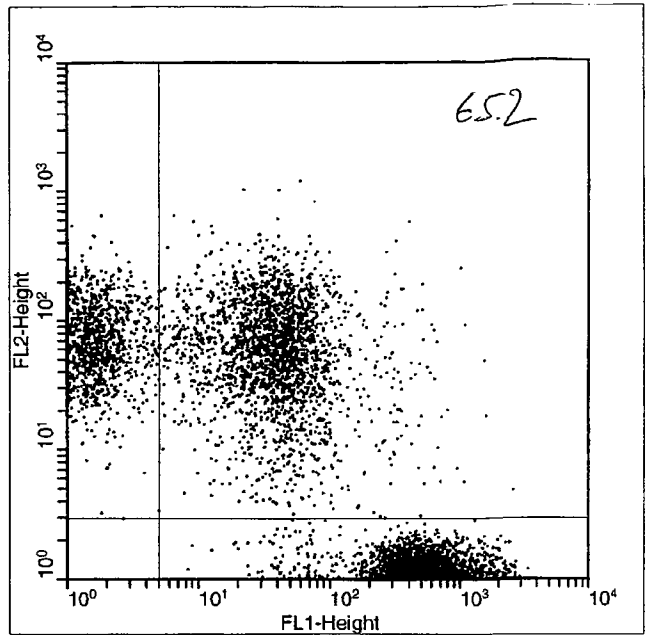
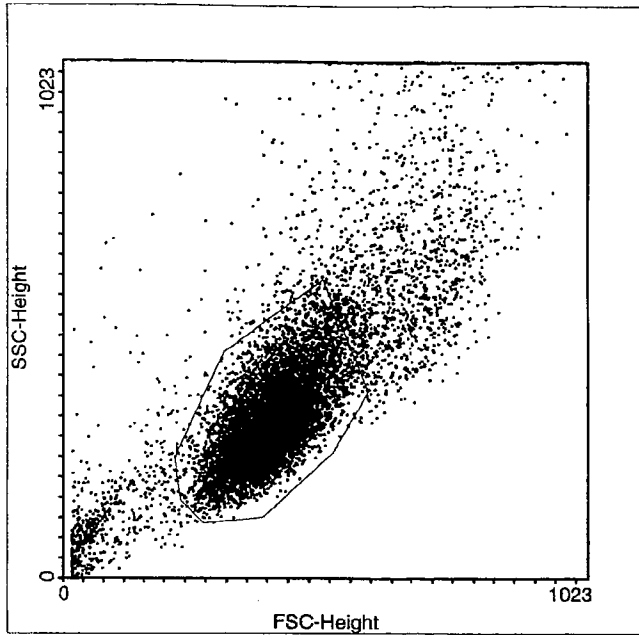
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:47:51 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA\*/DiI 9Gy 8h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 15677 78.39%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.8	35.2	2712	13.56	17.30
2 UR	26.6	26.5	4291	21.46	27.37
3 LL	2.7	2.1	5	0.03	0.03
4 LR	423.6	1.1	8669	43.35	55.30

} 44.67 → 61.3

$A/D = 0.81$



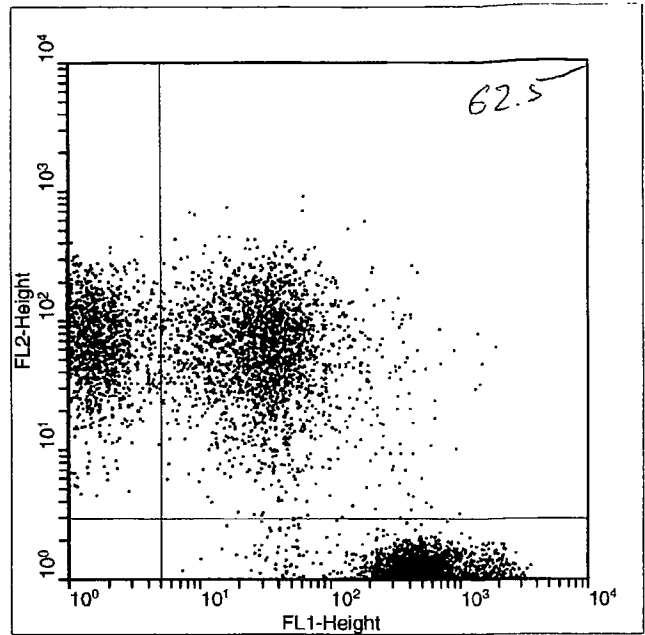
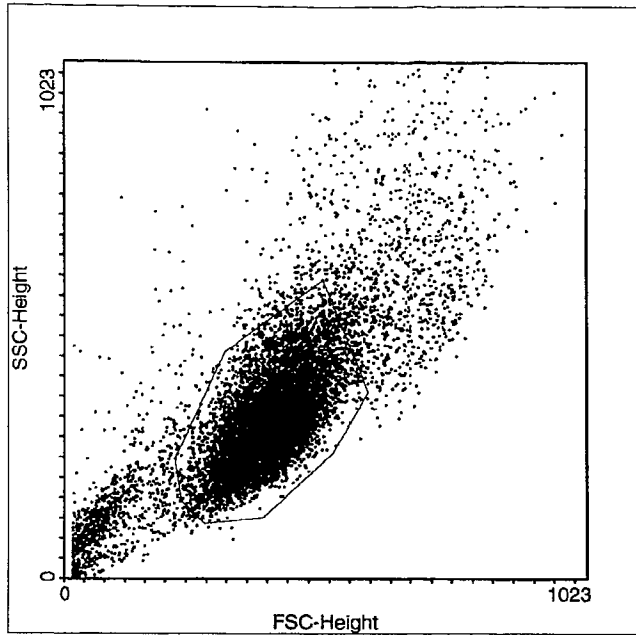
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:48:26 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA/DiI\* 9Gy 8h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 15852 79.26%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	69.8	2352	11.76	14.84
2 UR	38.9	54.2	4415	22.08	27.85
3 LL	1.0	1.7	1	0.01	0.01
4 LR	535.8	1.1	9084	45.42	57.31

} 42.69 → 65.2

A/D = 0.74



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu Apr 25 10:49:04 2002  
 Gates: R1  
 Project: Experiment:  
 File: 042502 Sample: WB CA\*/DiI\* 9Gy 8h  
 Date: 25-Apr-2 Parameters: 7  
 Total Events 20000 Gated Events 15528 77.64%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 180,120

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	1.6	70.0	2744	13.72	17.67
2 UR	31.6	57.1	4563	22.82	29.39
3 LL	2.6	1.5	4	0.02	0.03
4 LR	545.4	1.1	8217	41.09	52.92

47.06 → 62.5

$$A/D = 0.89$$

RESULTS OF THE EXPERIMENT OF APRIL 25, 2002

① %

	4L	6L	8L
CA/DiI (0Gy)	44.6	54.8	61.2
CA*/DiI (96y)	48.0	53.2	61.3
CA/DiI* (96y)	52.8	55.8	65.2
CA*/DiI* (96y)	56.2	60.8	62.5

② A/D ratio

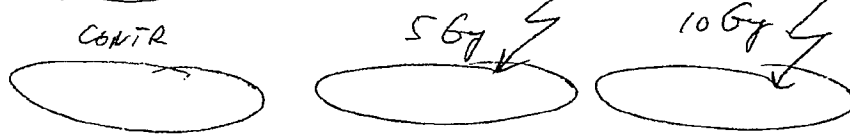
	0L	4L	6L	8L
CA/DiI (0Gy)	1.15	0.83	0.73	0.71
CA*/DiI (96y)	0.79	0.75	0.79	0.81
CA/DiI* (96y)	1.4	0.92	0.92	0.74
CA*/DiI* (96y)	0.97	1.01	0.93	0.89

DiI Studies

APRIL 26, 2002

DiI STUDIES

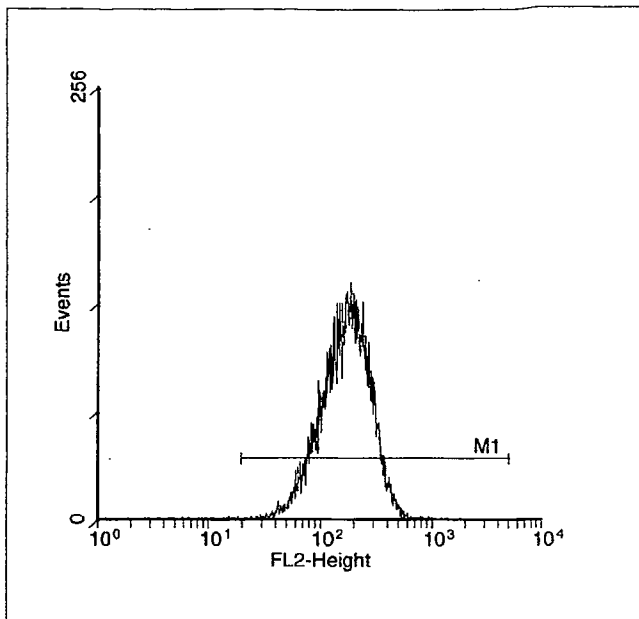
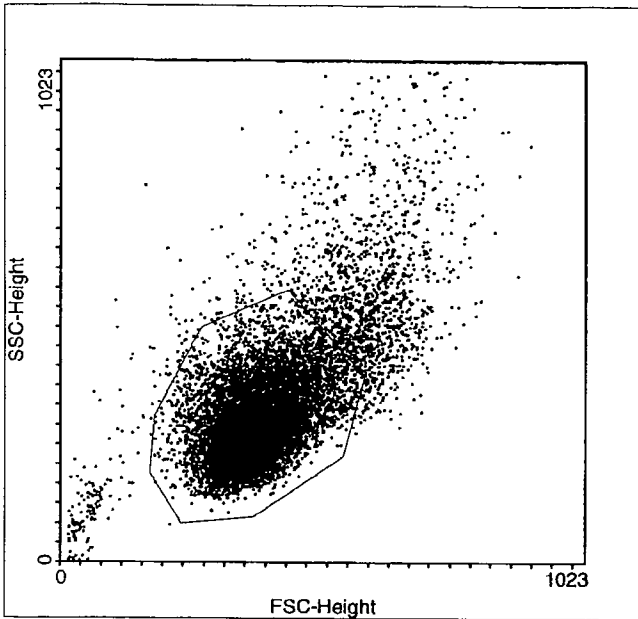
WB<sub>s</sub> p. 34  $\approx$  80% confluency



- Incubation  $\approx$  10 min after irradiation at  $+37^{\circ}\text{C}$ .
- Add 5  $\mu\text{l}$  of DiI to each culture
- Incubation  $\approx$  15 min at  $+37^{\circ}\text{C}$ .
- Aspirate medium
- Add New D-medium for (4 ml).
- Incubation  $\approx$  30 min at  $+37^{\circ}\text{C}$ .
- Trypsinization and harvesting into 3 plastic tubes for FCM.
- Fixation in  $\approx$  1.2% FA.
- FCM.

Note: Two sources of  $^{137}\text{Cs}$  = 688.65 Rad/min.

5 Gy  $\approx$  0.73 min  
10 Gy  $\approx$  1.45 min

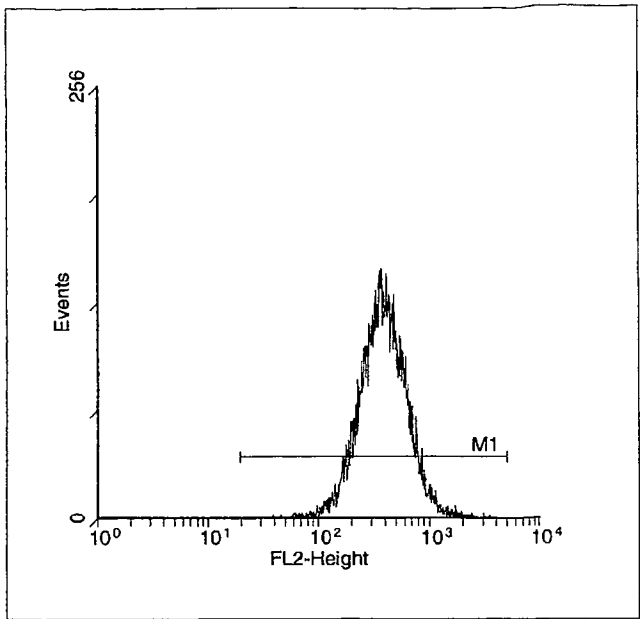
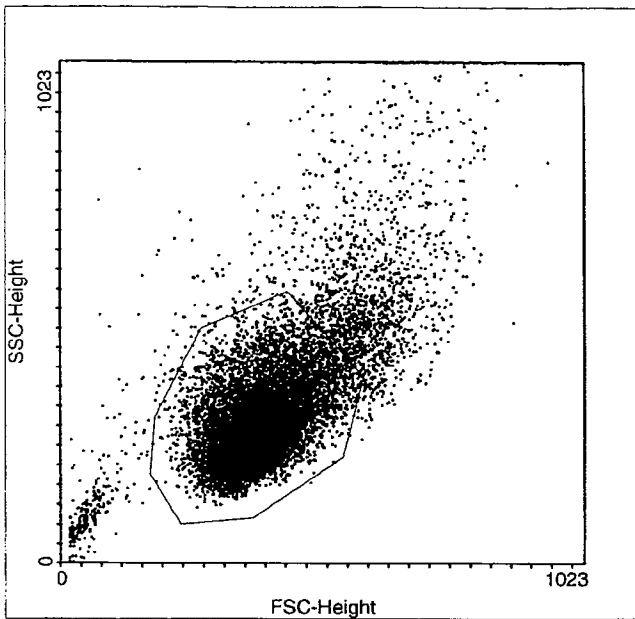


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 26 10:22:15 2002  
 Gates: R1

Project: Experiment:  
 File: 042602 Sample: WBS control (2.1.2. FA)  
 Date: 26-Apr-2 Parameters: 1

Total Events 20000 Gated Events 16855 84.28%  
 System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	16855	84.28	100.00	165.59	10.15	140,191.095
	1	331, 948	16829	84.15	99.85	<u>166.36</u>	9.87	140,191.095



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 26 10:26:43 2002

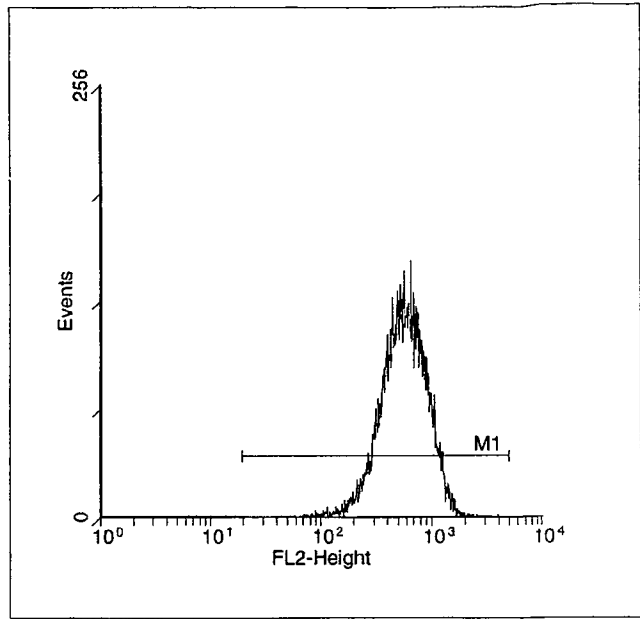
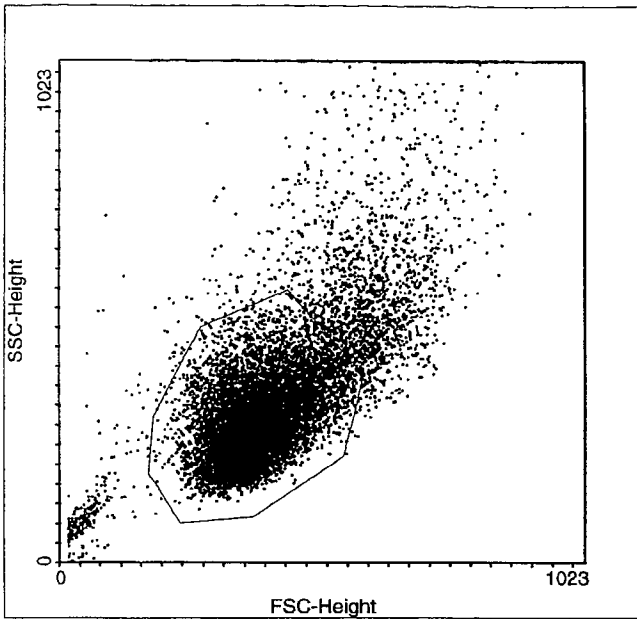
Gates: R1  
 Project: Experiment:  
 File: 042602 Sample: WBS DiI 5Gy *1.2i FA*

Date: 26-Apr-2 Parameters: 1  
 Total Events 20000 Gated Events 16693 83.46%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	16693	83.47	100.00	381.56	8.60	148,371.803
	1	331, 948	16681	83.41	99.93	382.56	8.44	148,371.803

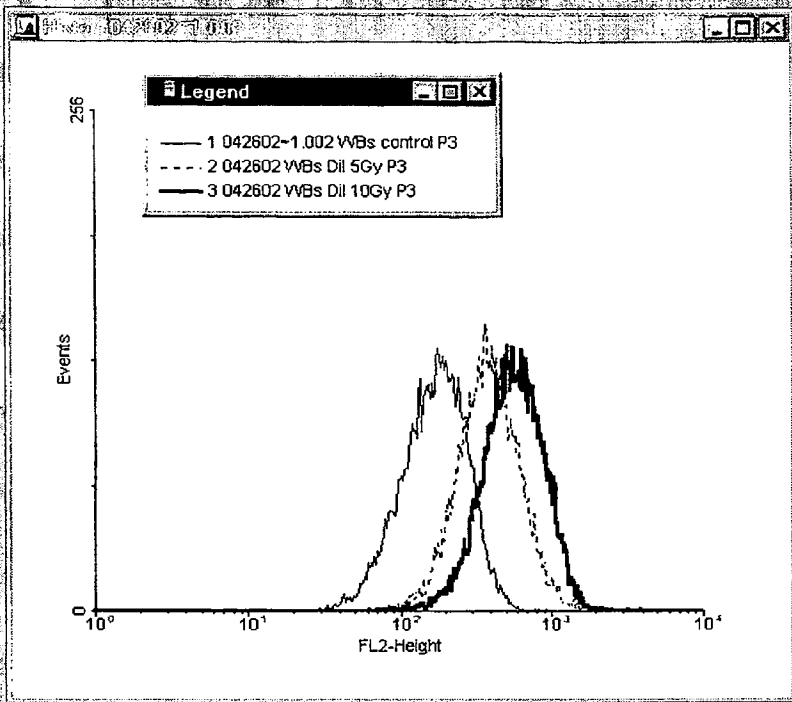




Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Apr 26 10:28:06 2002

Gates: R1  
 Project: Experiment:  
 File: 042602 Sample: WBS DiI 10Cy 1.2% FA  
 Date: 26-Apr-2 Parameters: 1  
 Total Events 20000 Gated Events 16224 81.12%  
 System: Log Parameter Means: Geometric

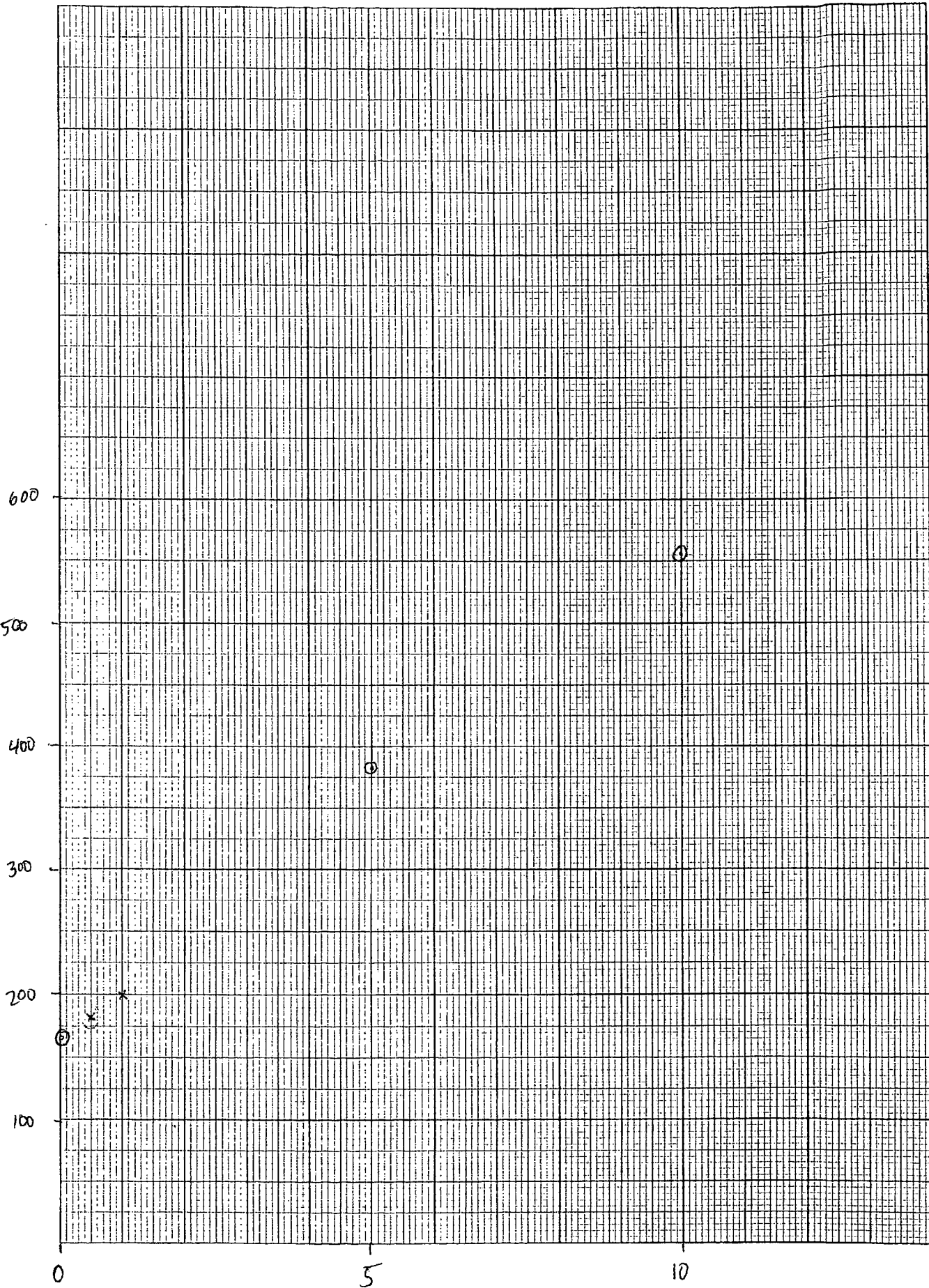
Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	16224	81.12	100.00	554.33	7.82	152,649.382
	1	331,948	16214	81.07	99.94	555.78	7.63	152,649.382



RESULTS OF <sup>EXP.</sup> APRIL 26, 2002

46 1240

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MAY 1, 2002

DI I STUDIES.

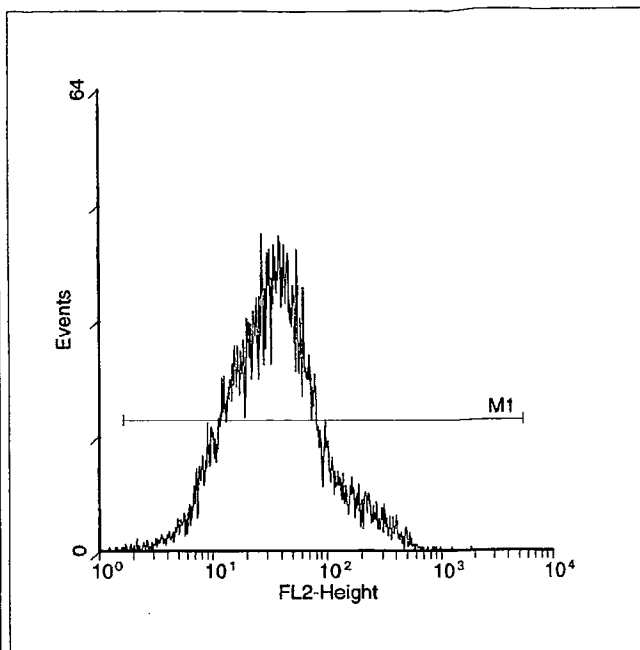
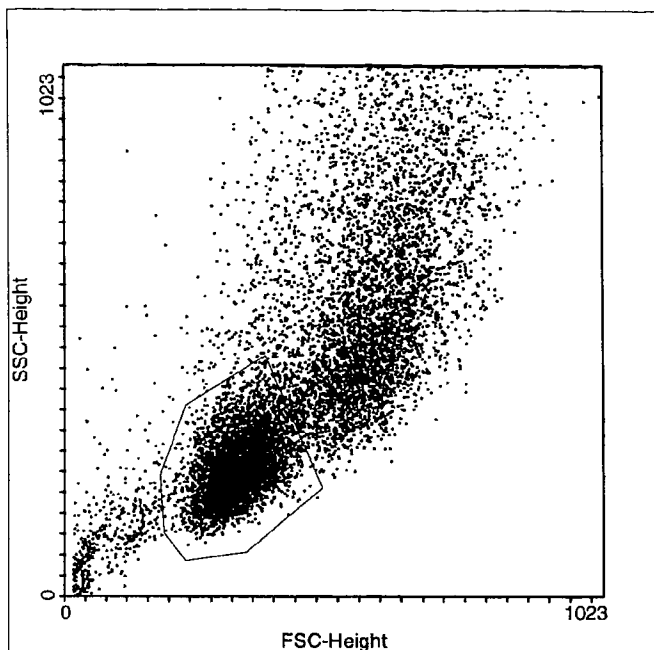
WB<sub>s</sub> (p. 34.)  $\approx 100\%$  confluency.

IRRADIATION AT 8:15.

DOSES: 0 Gy ; 0.5 Gy ; 1.0 Gy ; 5.0 Gy ; 10 Gy.

IRRADIATION WITH TWO SOURCES. RATE: 688.65 Rad/min

0.5 Gy	-	0.07 min.
1 Gy	-	0.14
5 Gy	-	0.73
10 Gy	-	1.45



Multiple Document Interface for Flow Cytometry

WinMDI Version 2.8 - Windows 3.95/DOS 7.10

Wed May 01 12:08:13 2002

Gates: R1

Project: Experiment:

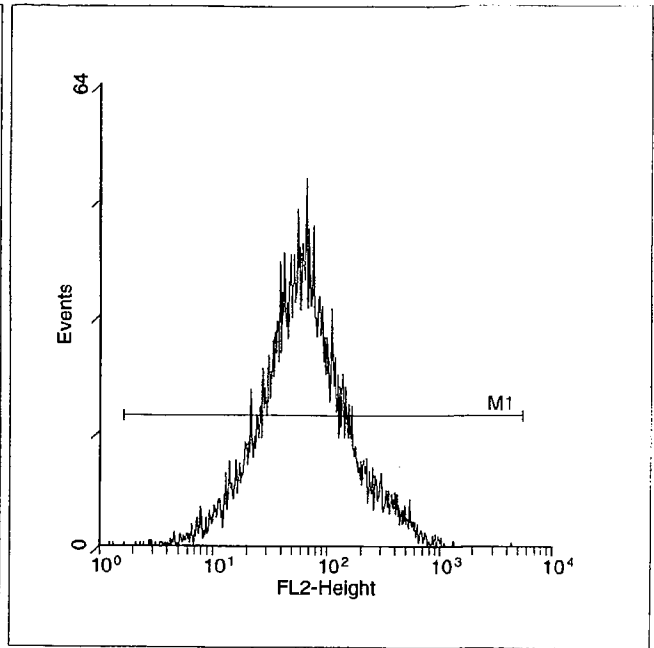
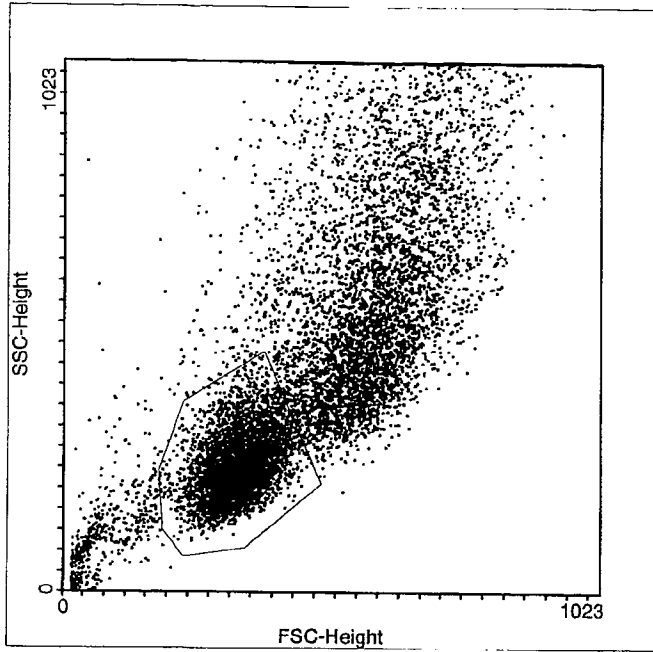
File: 053002 Sample: WB DiI Ocy

Date: 1-May-2 Parameters: 1

Total Events 20000 Gated Events 8883 44.42%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	8883	44.42	100.00	34.78	26.81	52,27.3842
	1	55,958	8878	44.39	99.94	34.84	26.72	52,27.3842



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 01 12:17:54 2002

Gates: R1

Project: Experiment:

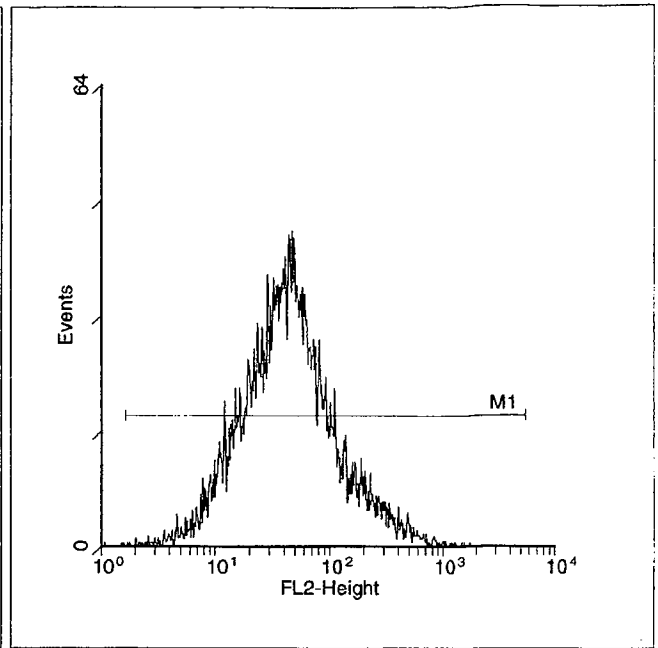
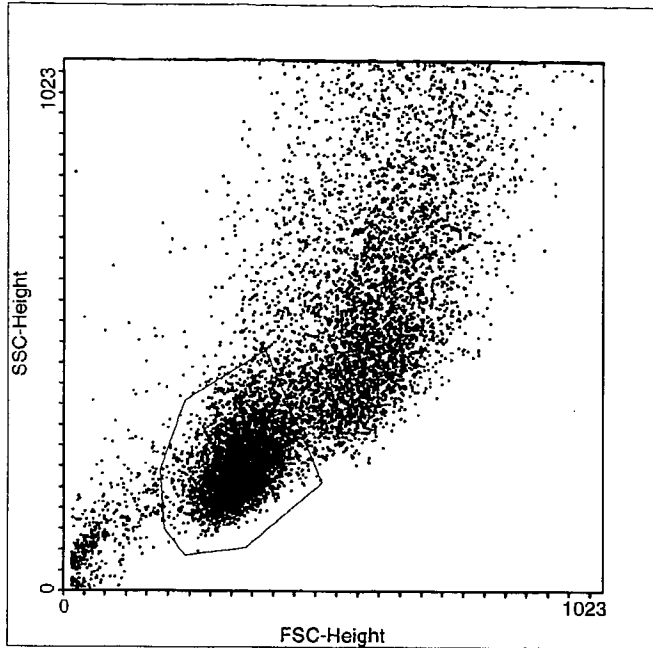
File: 053002 Sample: WB DiI 0.5Gy

Date: 1-May-2 Parameters: 1

Total Events 20000 Gated Events 8308 41.54%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	8308	41.54	100.00	62.87	21.99	56,69.1582
	1	55, 958	8306	41.53	99.98	62.92	21.94	56,69.1582



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 01 12:18:35 2002

Gates: R1

Project: Experiment:

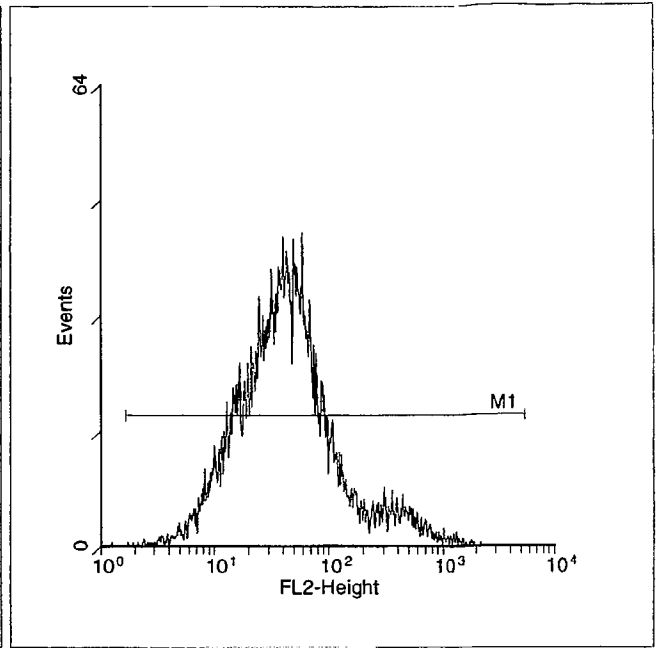
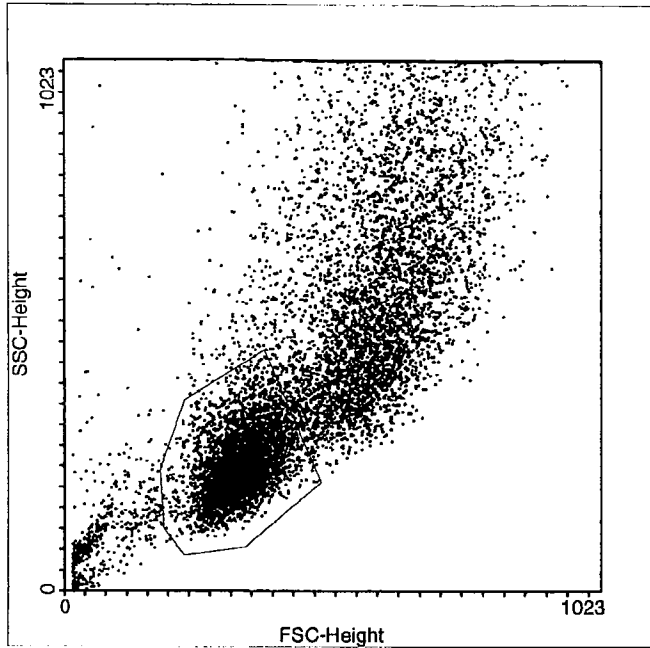
File: 053002 Sample: WB DiI 1Gy

Date: 1-May-2 Parameters: 1

Total Events 20000 Gated Events 8326 41.63%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	8326	41.63	100.00	43.76	25.34	53,47.8286
	1	55,958	8323	41.62	99.96	43.82	25.28	53,47.8286



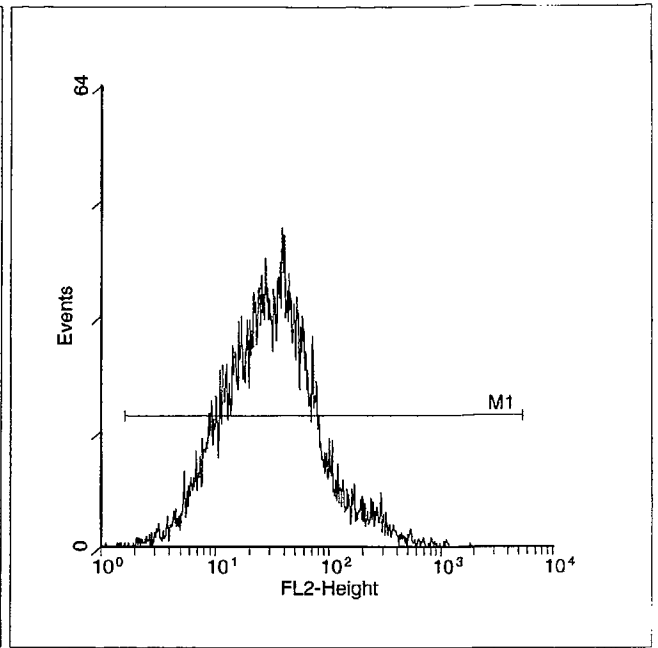
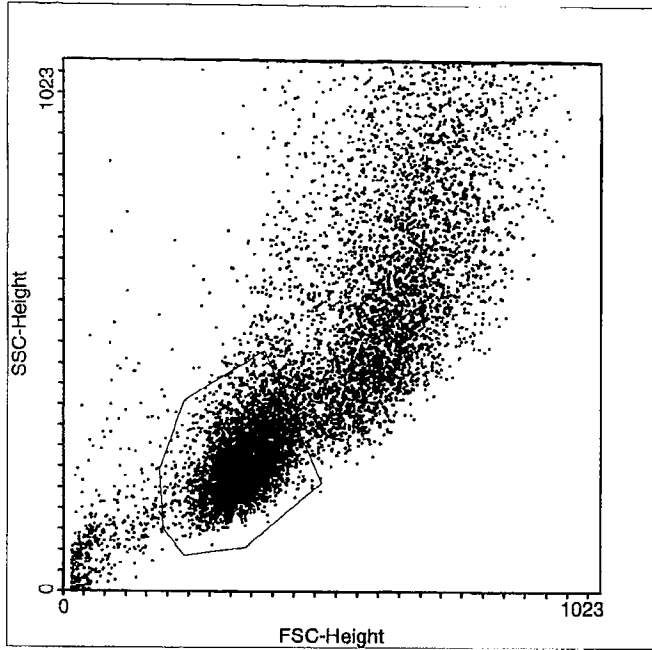
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 01 12:19:10 2002  
 Gates: R1

Project: Experiment:  
 File: 053002 Sample: WB DiI 5Gy  
 Date: 1-May-2 Parameters: 1

Total Events 20000 Gated Events 8397 41.99%  
 System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	8397	41.99	100.00	43.79	27.15	49,59.3523
	1	55,958	8396	41.98	99.99	43.81	27.13	49,59.3523





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 01 12:19:59 2002  
 Gates: R1

Project: Experiment:  
 File: 053002 Sample: WB DiI 10Gy  
 Date: 1-May-2 Parameters: 1

Total Events 20000 Gated Events 8750 43.75%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	8750	43.75	100.00	31.12	28.19	48,40.3152
	1	55, 958	8743	43.72	99.92	31.20	28.06	48,40.3152

MAY 2, 2002

DI STUDIES

WBs  $(p.35)$  at 60% confluency.

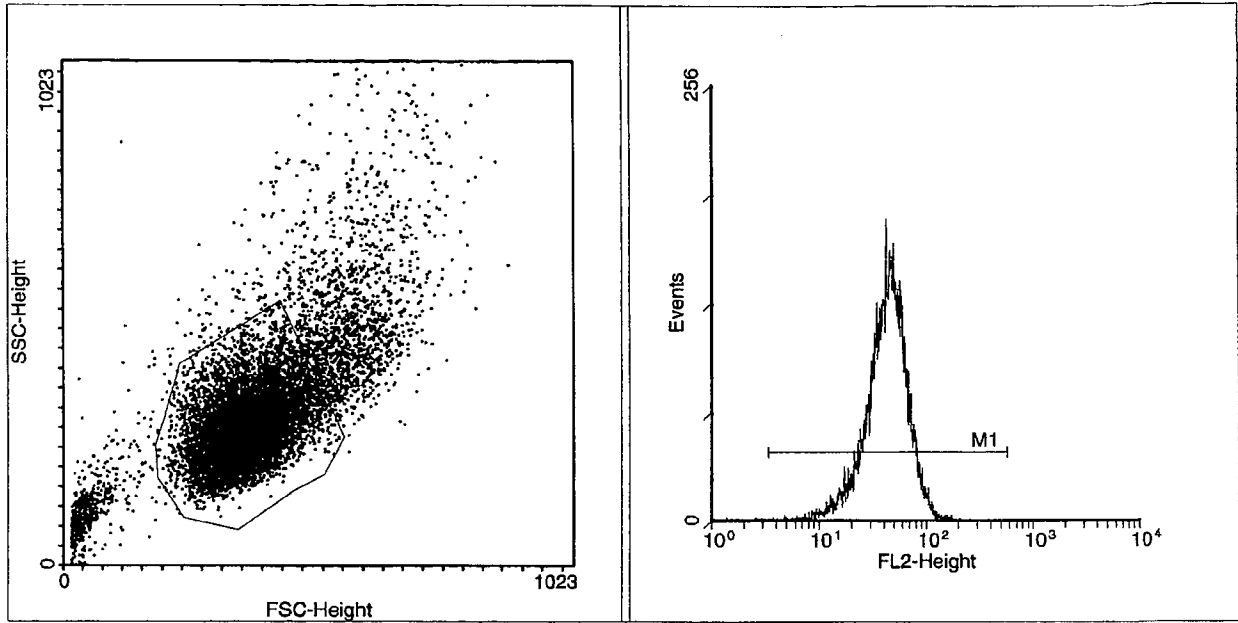
IRRADIATION AT 8:15

DOSES: 0 Gy : 0.5 Gy : 1 Gy : 5 Gy : 10 Gy.

IRRADIATION WITH TWO SOURCES: RATE: 688.65 Rad/min

0.5 Gy - 0.07 min  
1 Gy - 0.14  
5 Gy - 0.73  
10 Gy - 1.45

INCUBATION WITH THE STAIN: 30 min.  
(AFTER IRRADIATION)



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu May 02 11:42:45 2002

Gates: R1

Project: Experiment:

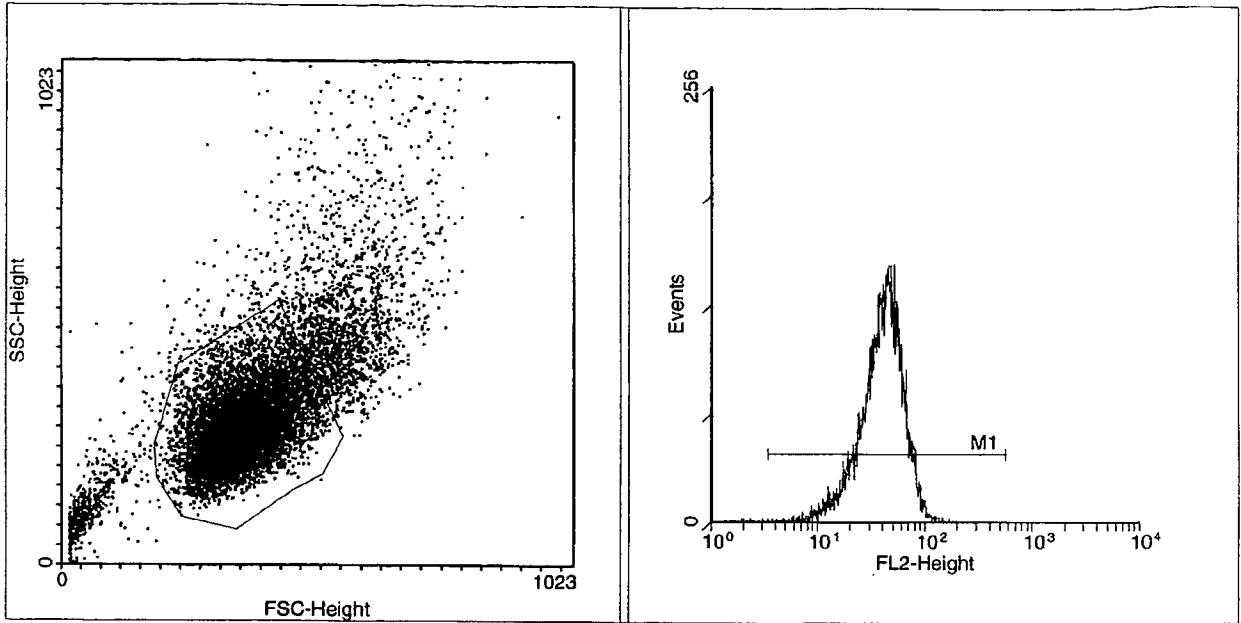
File: 050202 Sample: WB Ogy

Date: 2-May-2 Parameters: 1

Total Events 20000 Gated Events 15694 78.47%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	15694	78.47	100.00	41.11	13.83	177,42.5507
	1	136,706	15629	78.15	99.59	41.71	12.41	177,42.5507



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu May 02 11:44:31 2002

Gates: R1

Project: Experiment:

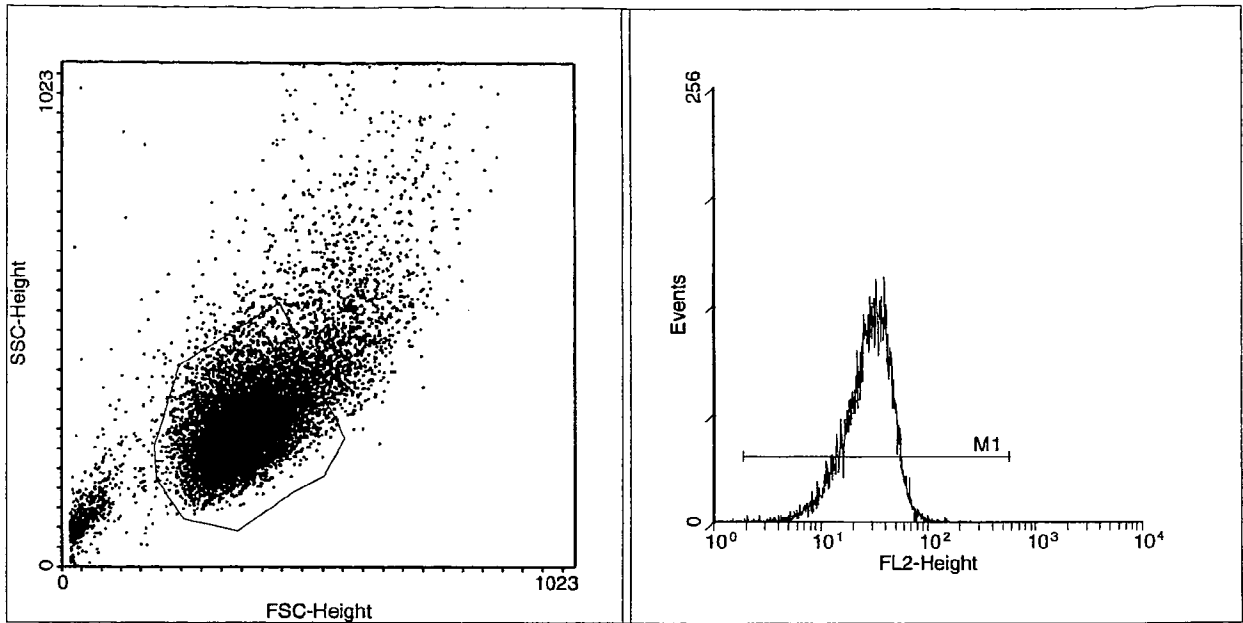
File: 050202 Sample: WB 0.5Gy

Date: 2-May-2 Parameters: 1

Total Events 20000 Gated Events 15454 77.27%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	15454	77.27	100.00	37.76	14.65	152,50.9368
	1	136, 706	15378	76.89	99.51	38.38	13.12	152,50.9368



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu May 02 11:45:25 2002

Gates: R1

Project: Experiment:

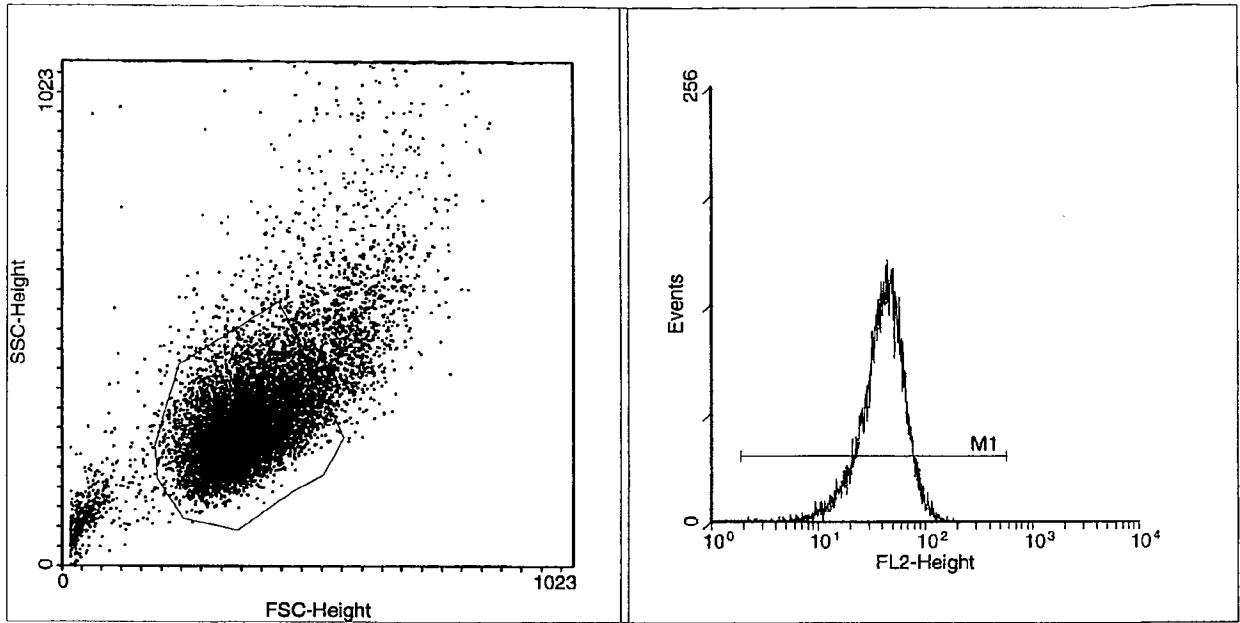
File: 050202 Sample: WB 1Gy

Date: 2-May-2 Parameters: 1

Total Events 20000 Gated Events 15831 79.15%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	15831	79.16	100.00	26.65	17.30	144,38.5423
	1	70,706	15762	78.81	99.56	27.03	16.01	144,38.5423



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu May 02 11:46:05 2002

Gates: R1

Project: Experiment:

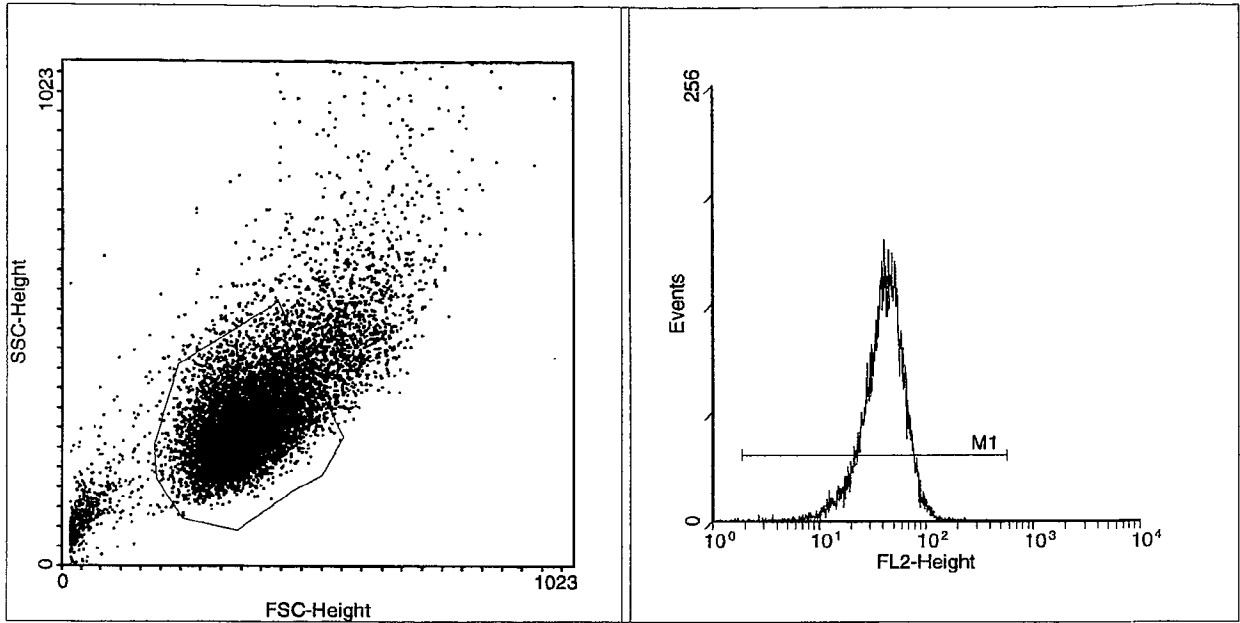
File: 050202 Sample: WB 5Gy

Date: 2-May-2 Parameters: 1

Total Events 20000 Gated Events 15927 79.64%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	15927	79.64	100.00	39.04	14.40	154,44.5079
	1	70, 706	15886	79.43	99.74	39.40	13.50	154,44.5079



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Thu May 02 11:46:26 2002

Gates: R1

Project: Experiment:

File: 050202 Sample: WB 10Gy

Date: 2-May-2 Parameters: 1

Total Events 20000 Gated Events 15827 79.14%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	15827	79.14	100.00	39.21	13.97	166,41.047
	1	70, 706	15786	78.93	99.74	39.58	13.04	166,41.047

DiL STUDIES

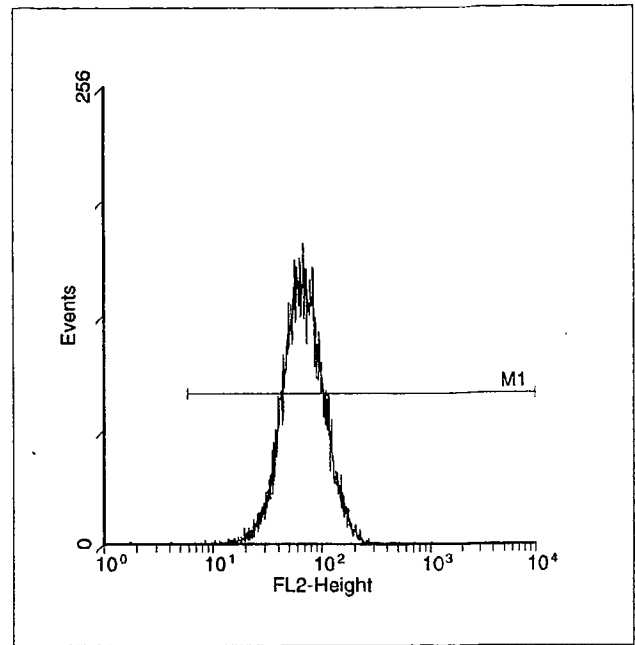
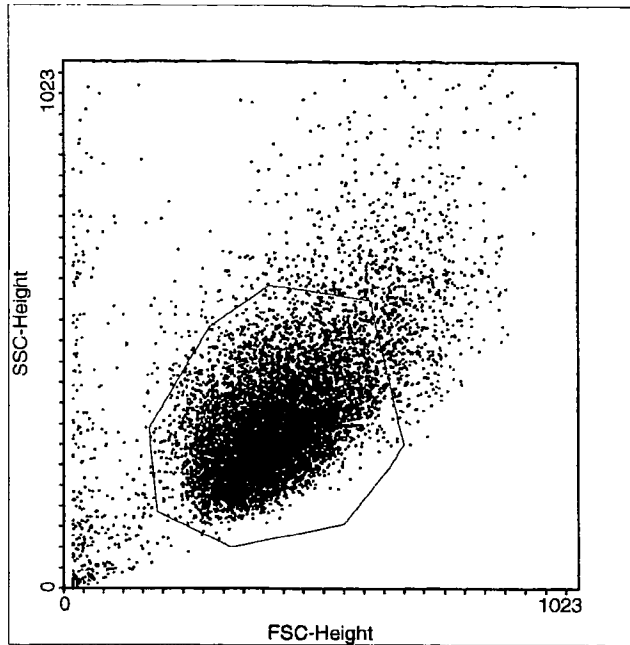
MAY 3, 2002

WB<sub>s</sub> (p. 35)  $\approx$  50% confluency

IRRADIATION WITH  $\alpha$ -PARTICLES : (5 min) AND (10 min).

INCUBATION WITH DiL :  $\approx$  15 min.  
(AFTER IRRADIATION)





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri May 03 11:57:07 2002

Gates: R1

Project: Experiment:

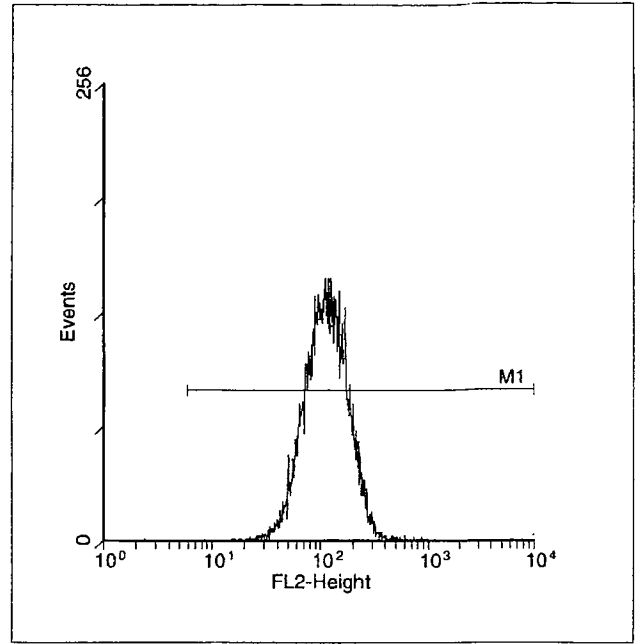
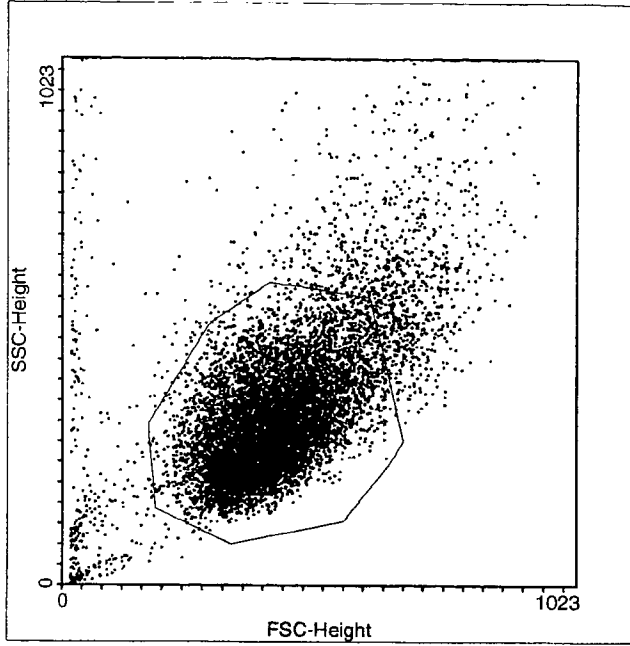
File: 050302 Sample: WB control (alpha)

Date: 3-May-2 Parameters: 1

Total Events 20000 Gated Events 17350 86.75%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	17350	86.75	100.00	67.54	10.53	168,68.539
	1	197,1023	17344	86.72	99.97	67.63	10.39	168,68.539



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri May 03 11:58:54 2002

Gates: R1

Project: Experiment:

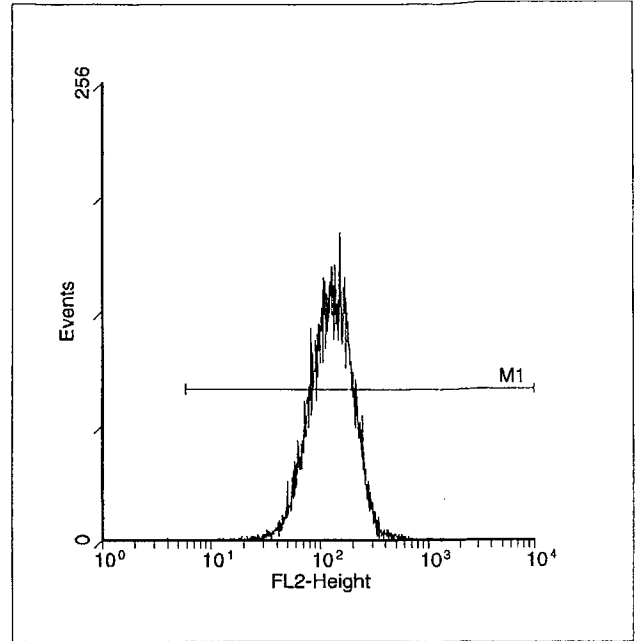
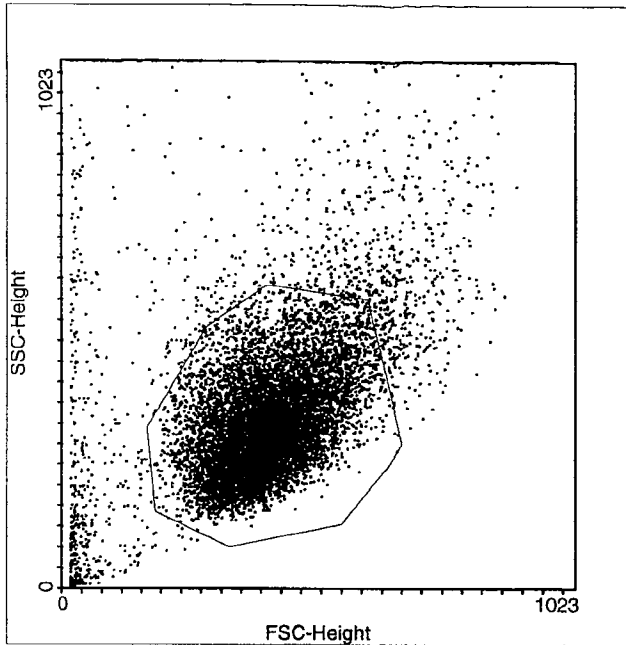
File: 050302 Sample: WB control (alpha 5 min)

Date: 3-May-2 Parameters: 1

Total Events 20000 Gated Events 17222 86.11%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	17222	86.11	100.00	111.65	9.59	147,125.215
	1	197,1023	17221	86.11	99.99	111.67	9.57	147,125.215



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri May 03 11:59:24 2002

Gates: R1

Project: Experiment:

File: 050302 Sample: WB control (alpha 10 min)

Date: 3-May-2 Parameters: 1

Total Events 20000 Gated Events 17345 86.72%

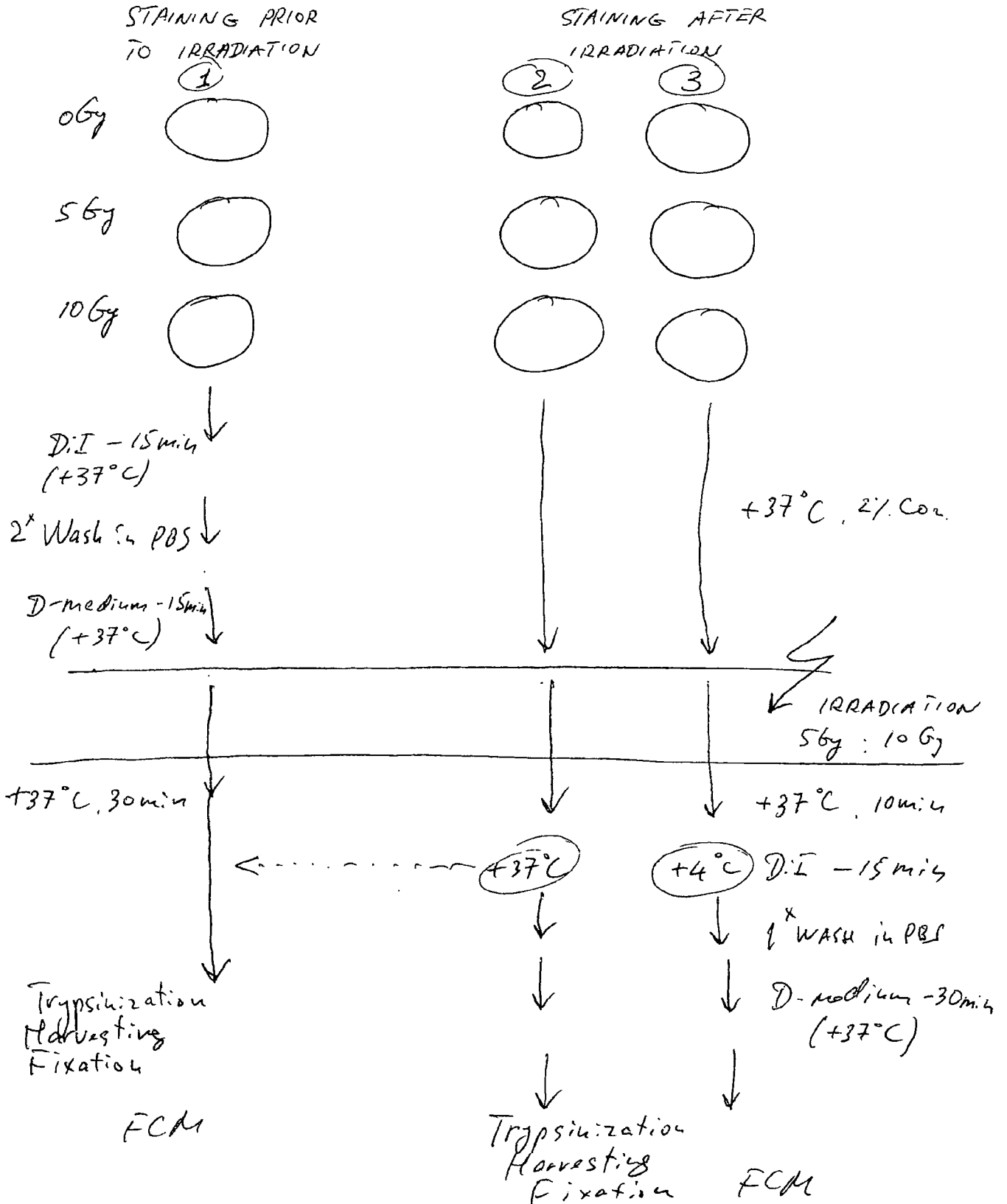
System: Log Parameter Means: Geometric

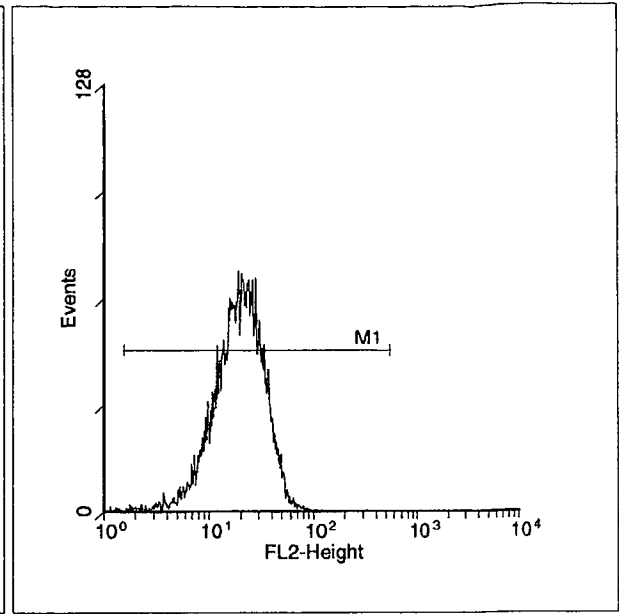
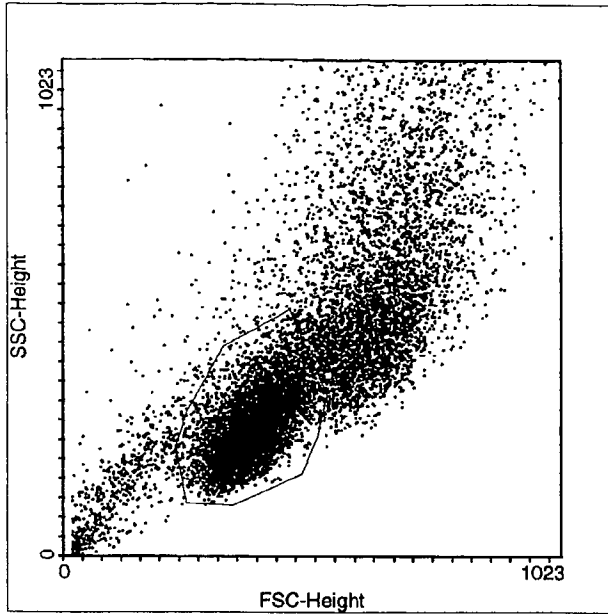
Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	17345	86.72	100.00	<u>125.44</u>	9.47	172,152.614
	1	197,1023	17344	86.72	99.99	<u>125.47</u>	9.46	172,152.614

D:1 STUDIES

MAY 6, 2002

WB<sub>5</sub> (p. 36) ~ 100% confluency





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 06 11:39:35 2002

Gates: R1

Project: Experiment:

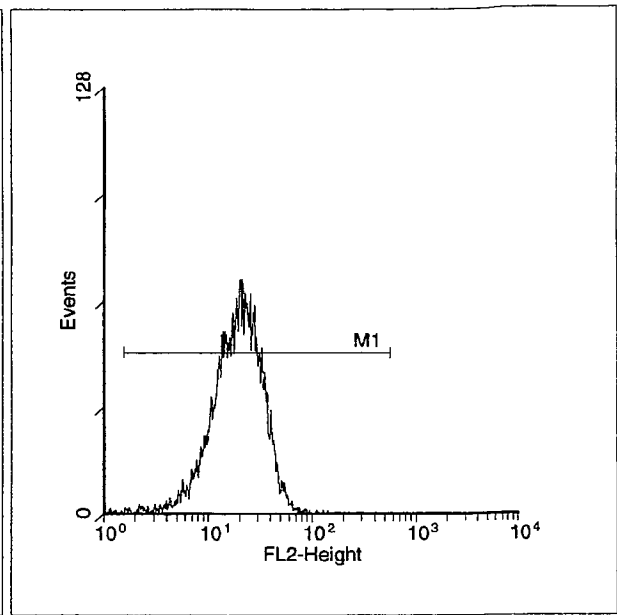
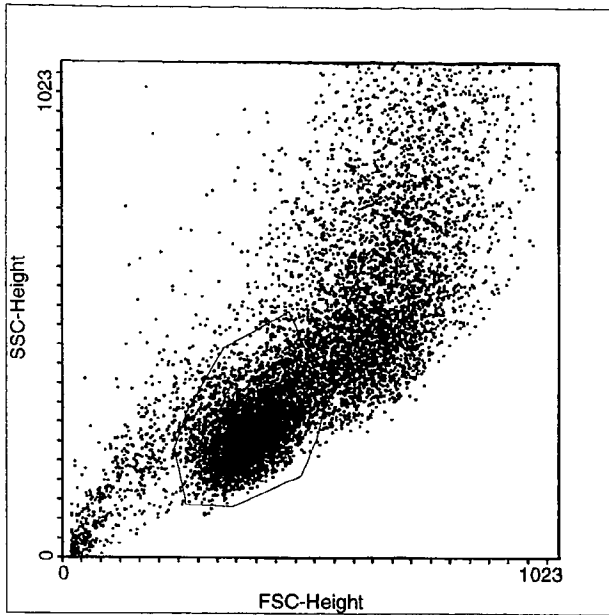
File: 050602 Sample: WB-1 Ogy

Date: 6-May-2 Parameters: 1

Total Events 20000 Gated Events 9852 49.26%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	9852	49.26	100.00	18.16	23.35	160,1
	1	50, 703	9675	48.38	98.20	19.14	18.93	82,28.3874



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 06 11:41:54 2002

Gates: R1

Project: Experiment:

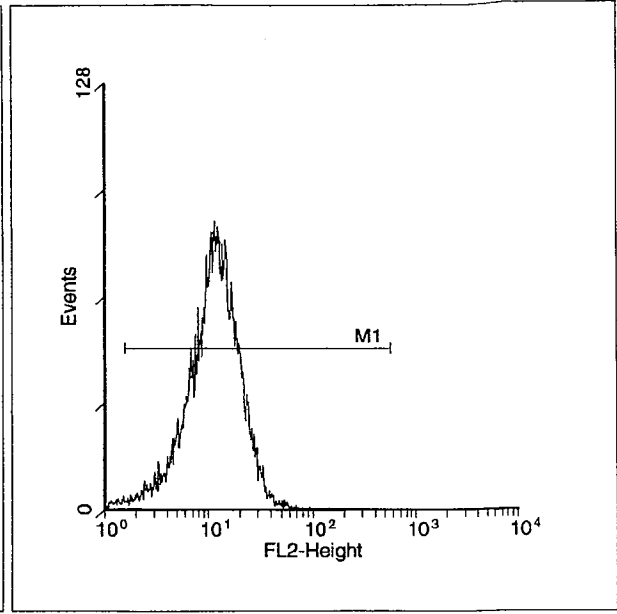
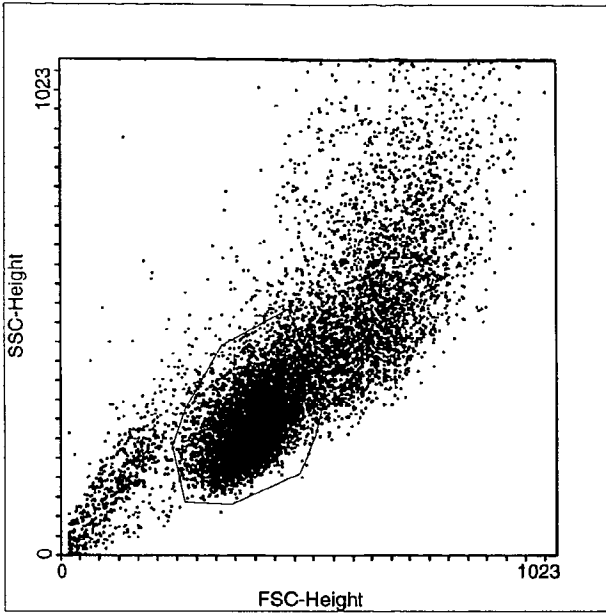
File: 050602 Sample WB-1 5Gy

Date: 6-May-2 Parameters: 1

Total Events 20000 Gated Events 9344 46.72%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	9344	46.72	100.00	17.78	23.92	154,1
	1	50,703	9171	45.86	98.15	<u>18.77</u>	19.48	78,25.483



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 06 11:42:42 2002

Gates: R1

Project: Experiment

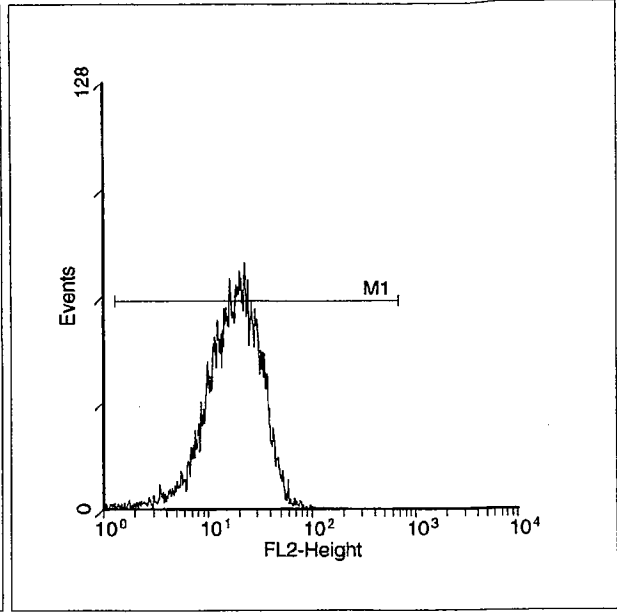
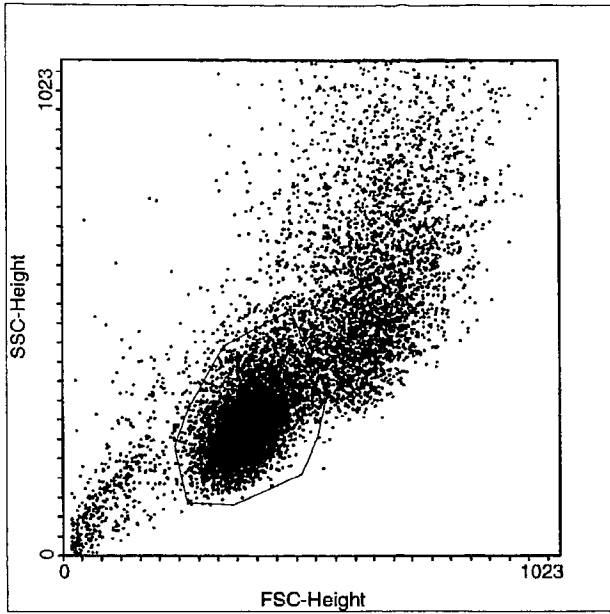
File: 050602 Sample WB-1 10Gy

Date: 6-May-2 Parameters: 1

Total Events 20000 Gated Events 11671 58.35%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	11671	58.36	100.00	10.09	31.90	364,1
	1	50, 703	11211	56.06	96.06	<u>11.07</u>	24.53	99,11.4444



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 06 13:13:47 2002

Gates: R1

Project: Experiment:

File: 050602 Sample: WB-2 0Gy

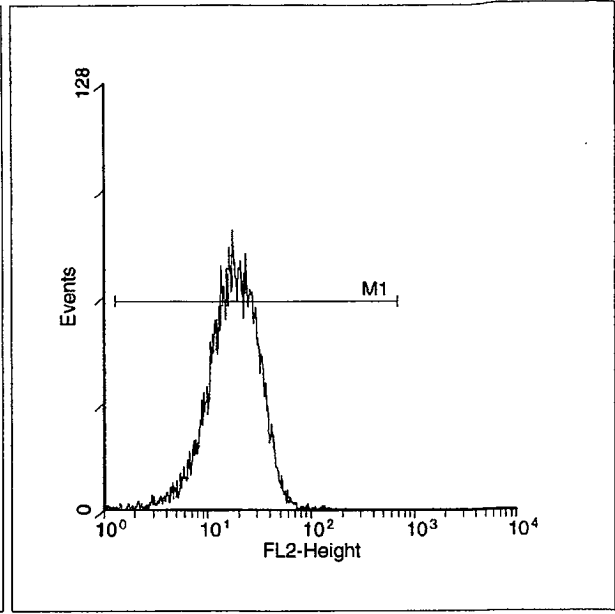
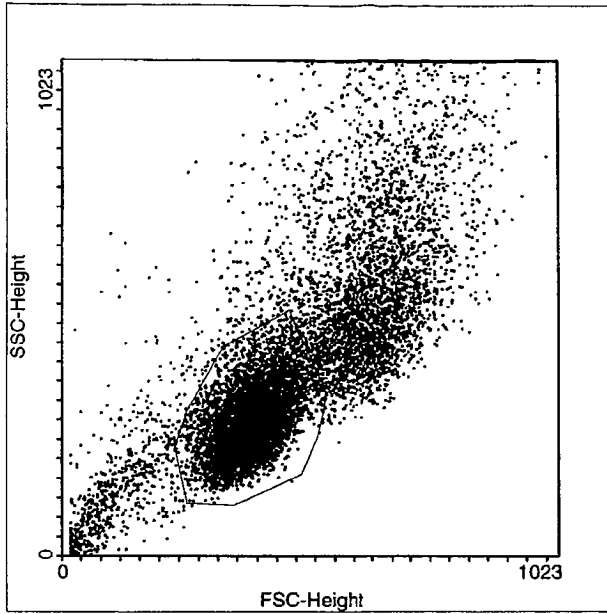
Date: 6-May-2 Parameters: 1

Total Events 20000 Gated Events 10813 54.06%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	10813	54.07	100.00	15.83	28.09	318,1
	1	28, 725	10475	52.38	96.87	17.30	21.30	83,19.9885





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 06 13:14:16 2002

Gates: R1

Project: Experiment:

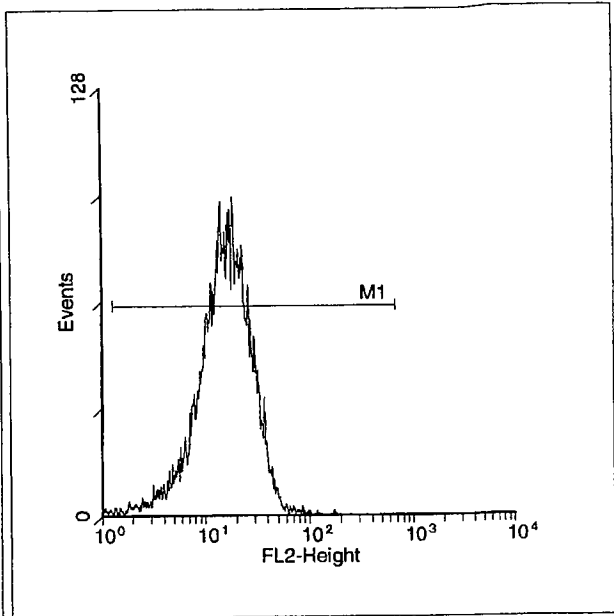
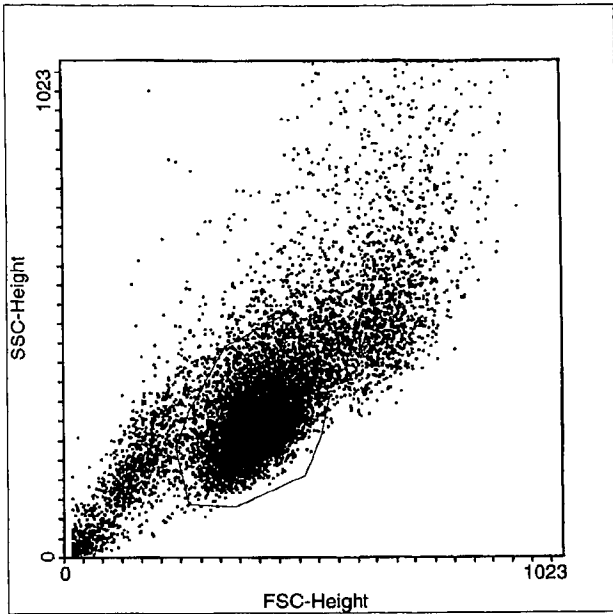
File: 050602 Sample: WB-2 5Gv

Date: 6-May-2 Parameters: 1

Total Events 20000 Gated Events 11411 57.06%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	11411	57.06	100.00	15.88	27.55	352,1
	1	28, 725	11043	55.22	96.78	17.41	20.33	91,16.2531



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 06 13:14:57 2002

Gates: R1

Project: Experiment:

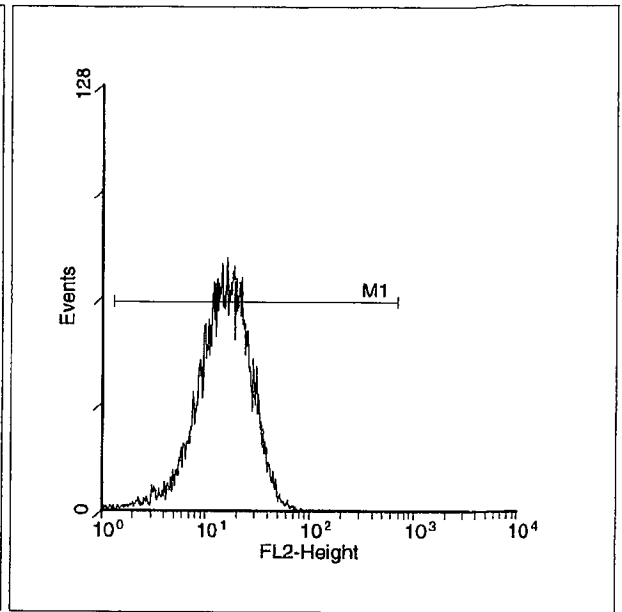
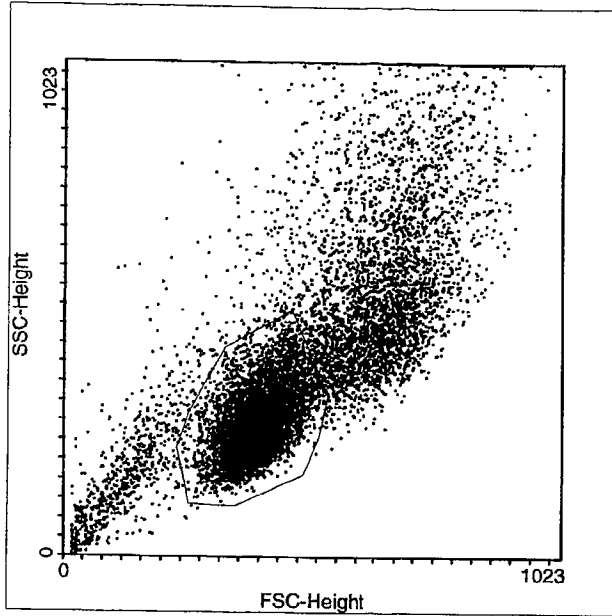
File: 050602 Sample: WB-2 10Gy

Date: 6-May-2 Parameters: 1

Total Events 20000 Gated Events 13187 65.93%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	13187	65.94	100.00	13.56	31.08	509,1
	1	28, 725	12645	63.22	95.89	15.16	22.76	101,18.7688



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 06 13:15:15 2002

Gates: R1

Project: Experiment:

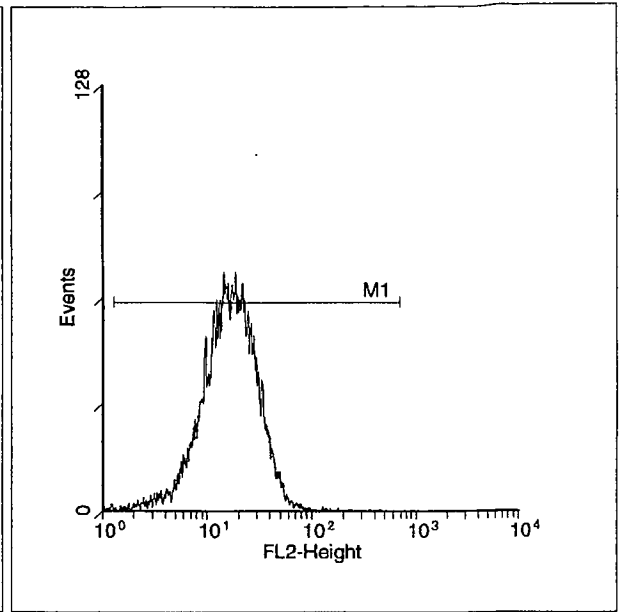
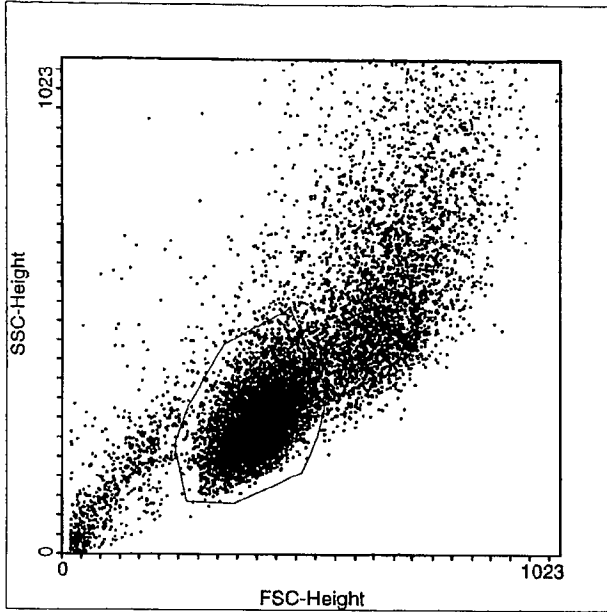
File: 050602 Sample: WB-3 0Gy

Date: 6-May-2 Parameters: 1

Total Events 20000 Gated Events 11034 55.17%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	11034	55.17	100.00	13.67	28.40	282,1
	1	28, 725	10726	53.63	97.21	14.74	22.52	81,12.079



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 06 13:15:35 2002

Gates: R1

Project: Experiment:

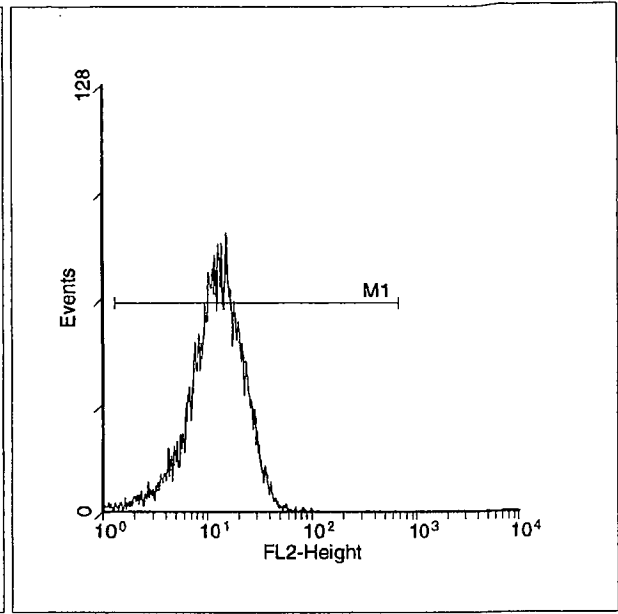
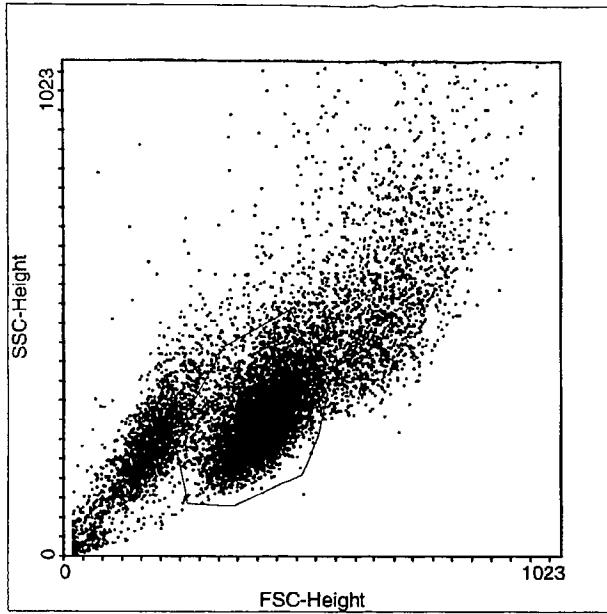
File: 050602 Sample: WB-3 5Gy

Date: 6-May-2 Parameters: 1

Total Events 20000 Gated Events 10824 54.12%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	10824	54.12	100.00	15.20	27.00	210,1
	1	28, 725	10590	52.95	97.84	16.14	22.35	77,15.1247



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 06 13:16:07 2002

Gates: R1

Project: Experiment:

File: 050602 Sample: WB-3 10Gy

Date: 6-May-2 Parameters: 1

Total Events 20000 Gated Events 11550 57.75%

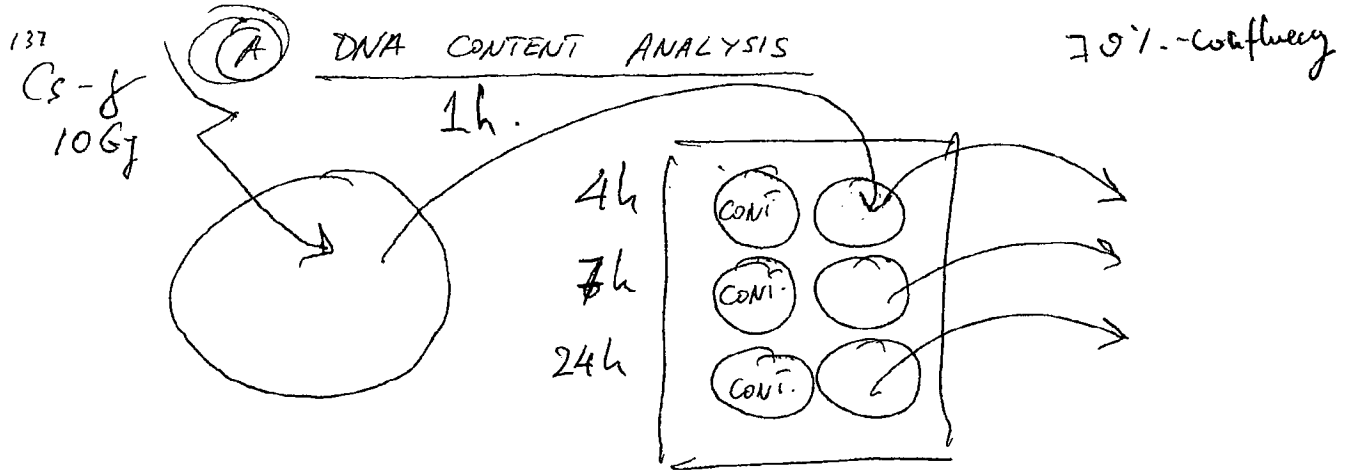
System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	11550	57.75	100.00	10.96	32.31	422,1
	1	28, 725	11091	55.46	96.03	12.10	24.66	88,15.3993

BYSTANDER EFFECTS  
(SUPERNATANT TRANSFER)

MAY 7, 2002

BYSTANDER EFFECTS (SUPERNATANT TRANSFER)



IRRADIATION TIME: 8:20

INCUBATION (+37°C: 2% ~~CO<sub>2</sub>~~ 8:25

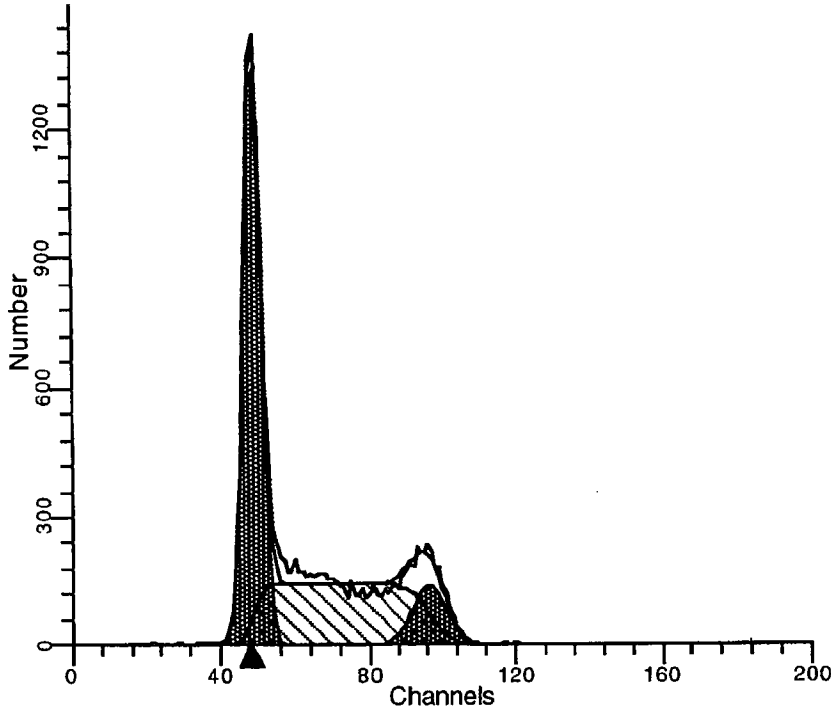
MEDIUM TRANSFER: 9:30 - OK

HARVESTING: 13:30 (4h) - OK

16:30 (7h) - OK

Next day 9:30 (24h).

NOTE: NO EFFECTS.



Date acquired: 8-May-2  
 File: 050802 .001  
 Source: WB Ogy 40M  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

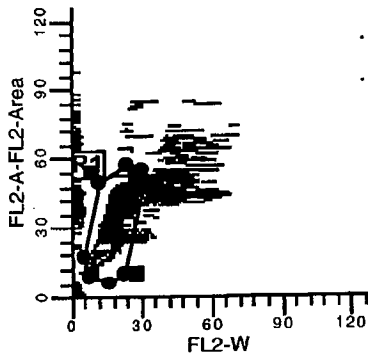
DIPLOID: 100.00 %  
 Dip G0-G1: 48.19 % at 48.64  
 Dip G2-M: 9.76 % at 96.54  
 Dip S: 42.04 % G2/G1: 1.98  
 Dip %CV: 4.70

Total S-Phase: 42.04 %

Extra Pop: %  
 Debris: 0.60 %  
 Aggregates: 0.00 %  
 Modeled Events: 16432  
 RCS: 1.448

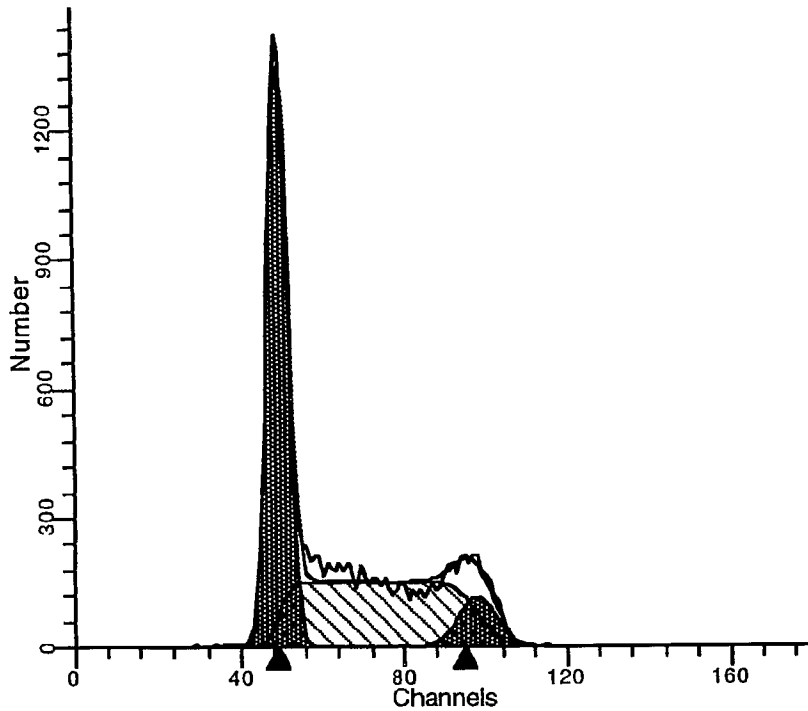
Diploid B.A.D.: 0.30 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFILT V2.0(PMac)





Date acquired: 8-May-2  
 File: 050802 .004  
 Source: WB 10Gy 4h  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

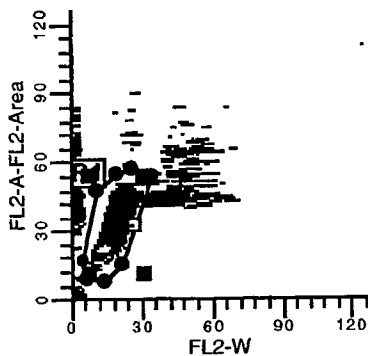
DIPLOID: 100.00 %  
 Dip G0-G1: 47.41 % at 49.27  
 Dip G2-M: 8.02 % at 98.01  
 Dip S: 44.57 % G2/G1: 1.99  
 Dip %CV: 4.69

Total S-Phase: 44.57 %

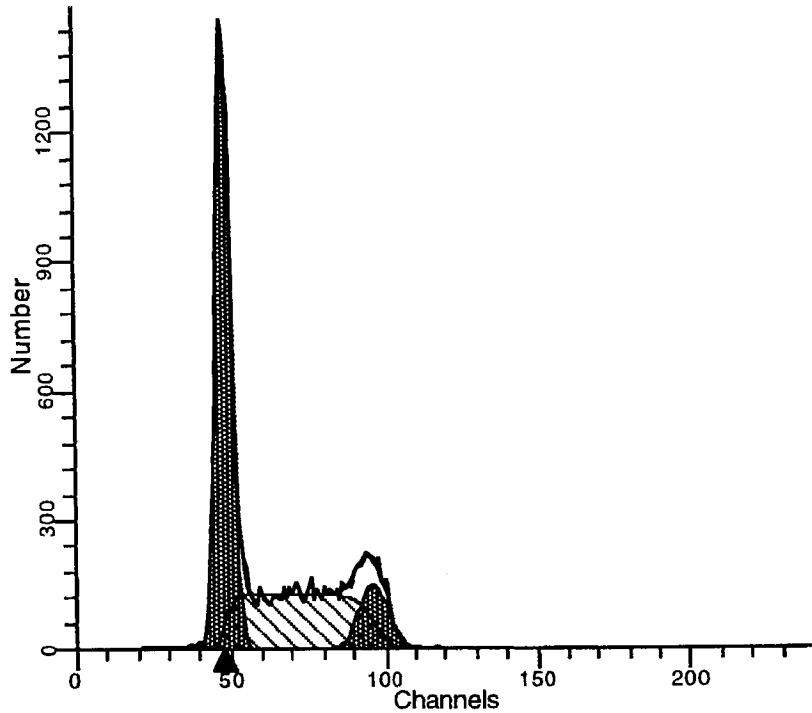
Extra Pop: %  
 Debris: 0.57 %  
 Aggregates: 0.00 %  
 Modeled Events: 16867  
 RCS: 1.699

Diploid B.A.D.: 0.28 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 8-May-2  
 File: 050802 .003  
 Source: WB 0Gy 7h  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

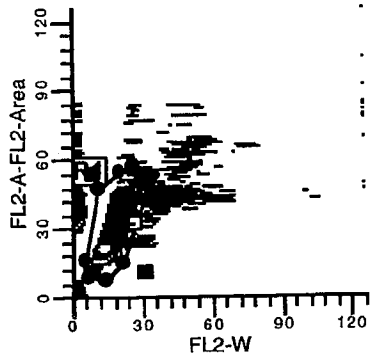
DIPLOID: 100.00 %  
 Dip G0-G1: 51.68 % at 48.25  
 Dip G2-M: 10.81 % at 96.20  
 Dip S: 37.51 % G2/G1: 1.99  
 Dip %CV: 4.83

Total S-Phase: 37.51 %

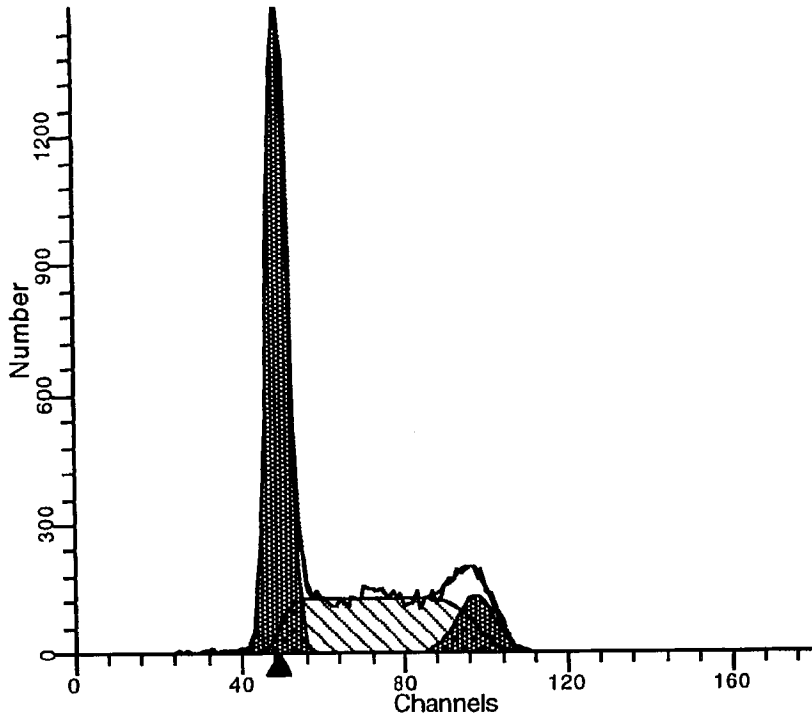
Extra Pop: %  
 Debris: 0.99 %  
 Aggregates: 0.00 %  
 Modeled Events: 16294  
 RCS: 0.723

Diploid B.A.D.: 0.51 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 8-May-2  
 File: 050802 .005  
 Source: WB 10Gy 7h  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

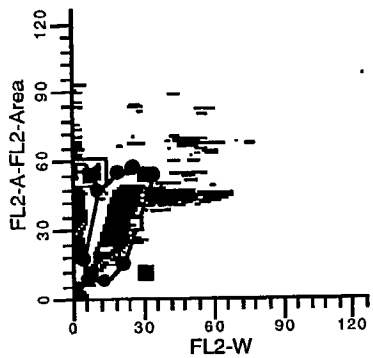
DIPLOID: 100.00 %  
 Dip G0-G1: 53.88 % at 49.26  
 Dip G2-M: 9.35 % at 97.55  
 Dip S: 36.77 % G2/G1: 1.98  
 Dip %CV: 4.67

Total S-Phase: 36.77 %

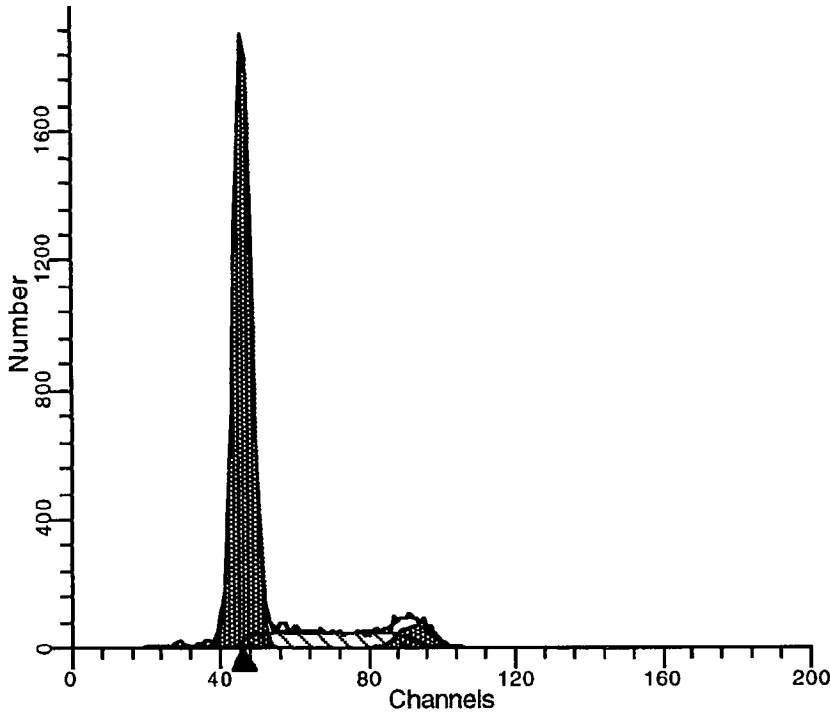
Extra Pop: %  
 Debris: 0.85 %  
 Aggregates: 0.00 %  
 Modeled Events: 16635  
 RCS: 1.129

Diploid B.A.D.: 0.40 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 8-May-2  
 File: 050802 .006  
 Source: WB 0Gy 24h  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

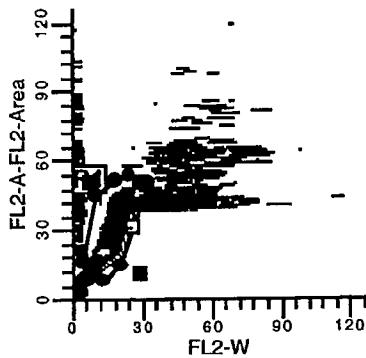
DIPLOID: 100.00 %  
 Dip G0-G1: 77.76 % at 46.23  
 Dip G2-M: 5.74 % at 92.61  
 Dip S: 16.49 % G2/G1: 2.00  
 Dip %CV: 4.99

Total S-Phase: 16.49 %

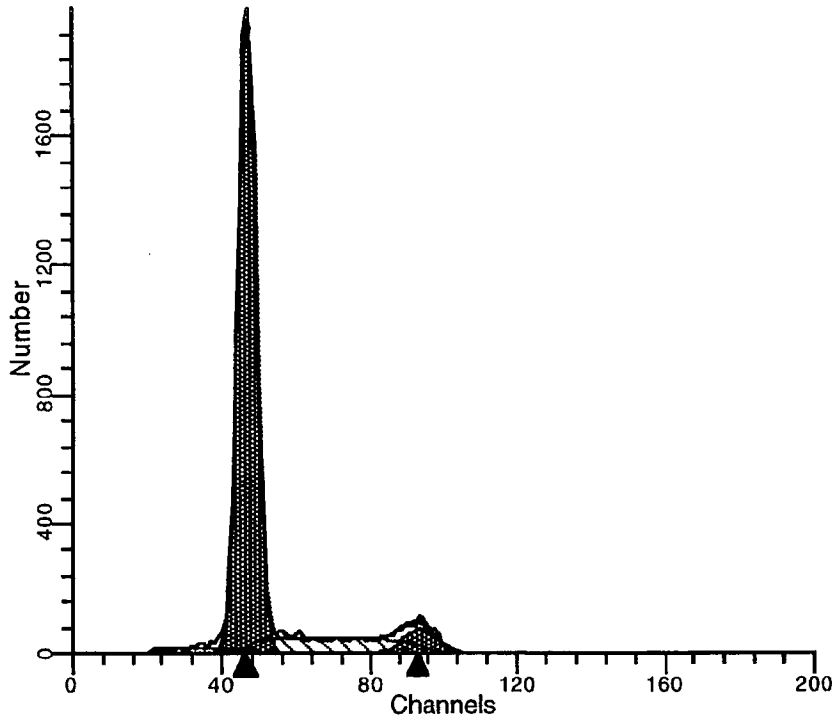
Extra Pop: %  
 Debris: 2.56 %  
 Aggregates: 0.00 %  
 Modeled Events: 14537  
 RCS: 1.137

Diploid B.A.D.: 0.91 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 8-May-2  
 File: 050802 .007  
 Source: WB 10Gy 24h  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

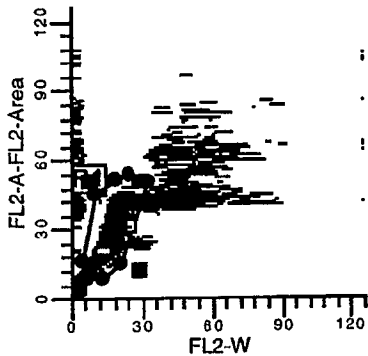
DIPLOID: 100.00 %  
 Dip G0-G1: 79.68 % at 46.81  
 Dip G2-M: 5.86 % at 93.61  
 Dip S: 14.46 % G2/G1: 2.00  
 Dip %CV: 4.80

Total S-Phase: 14.46 %

Extra Pop: %  
 Debris: 3.10 %  
 Aggregates: 0.00 %  
 Modeled Events: 14819  
 RCS: 1.478

Diploid B.A.D.: 1.07 % no aggs

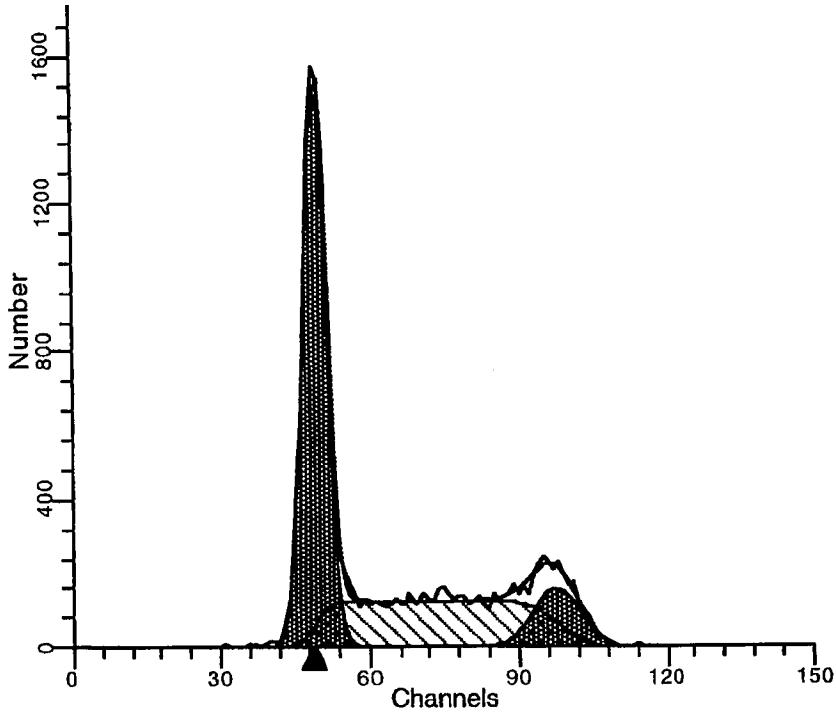
S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)

DNA CONTENT ANALYSIS

	G <sub>0</sub> /G <sub>1</sub>	S	G <sub>2</sub> /M
0 Gy 4h	48.19	42.04	9.76
10 Gy 4h	47.41	44.57	8.02
0 Gy 7h	51.68	37.51	10.81
10 Gy 7h	53.88	36.77	9.35
0 Gy 24h	77.76	16.49	5.74
10 Gy 24h	79.68	14.46	5.86



Date acquired: 8-May-2  
 File: 050802 .002  
 Source: ~~WB 10Gy in Fl~~  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

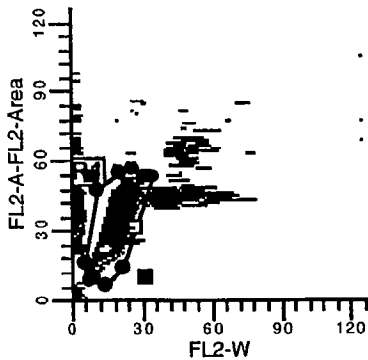
DIPLOID: 100.00 %  
 Dip G0-G1: 52.77 % at 49.25  
 Dip G2-M: 10.83 % at 97.65  
 Dip S: 36.40 % G2/G1: 1.98  
 Dip %CV: 4.63

Total S-Phase: 36.40 %

Extra Pop: %  
 Debris: 0.57 %  
 Aggregates: 0.00 %  
 Modeled Events: 16721  
 RCS: 1.255

Diploid B.A.D.: 0.29 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFITLT V2.0(PMac)

Q-CULTURE  
DNA

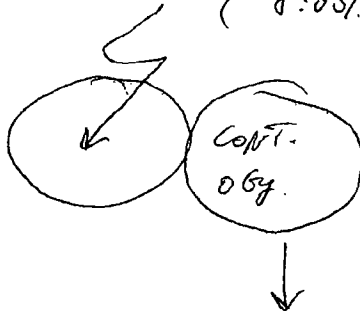


MAY 9, 2002

BYSTANDER EFFECTS

DNA content +  
CFDA staining

WBs p37 100% confluency  
137 (8:00)  
Cs-137 (5Gy)



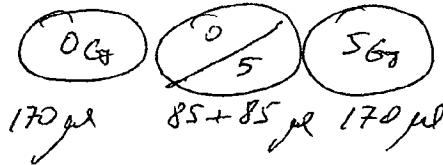
- prewarm 4ml CFDA SE staining - 20min at +37°C
- prewarm 4ml D-medium (Loading solution) (incubation 30min at +37°C)
- Plating: 10:00

Harvesting:  
(0.5ml Trypsin + 2ml medium)

Resusp.  
in 1ml  
D-medium



$$\begin{aligned} 3 \times 10^6 & - 1 \text{ ml} \\ 0.5 \times 10^6 & - x \\ x & = \frac{0.5 \times 10^6}{3 \times 10^6} = 0.17 \mu\text{l} \\ & \quad (170 \mu\text{l}) \end{aligned}$$



MAY 10, 2002

HARVESTING AND FIXATION IN METOX AND METOX/FA  
FCM.

- NOTE:
1. FA/MetOX fixation alters DNA content profile.
  2. No separation between labeled and non-labeled cells

MAY 13, 2002

$\Phi 5$  cm dish contains  $\approx 3 \times 10^6$  cells (100% confluency)  
 $S = \pi R^2$

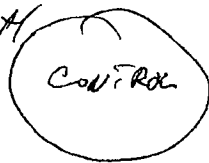
$$S_{\Phi 5} = 3.14 \times 2.5^2 = 3.14 \times 6.25 = 19.6 \text{ cm}^2$$

$$S_{\Phi 3.5} = 3.14 \times 1.75^2 = 3.14 \times 3.06 = 9.6 \text{ cm}^2$$

So,  $\Phi 3.5$  cm dish contains  $\approx 1.47 \times 10^6$  cells (100% confluency)

DNA content studies (BYSTANDER EFFECTS)

WB: 38p  
100% conflu



5 Gy ( $^{137}\text{Cs}$ )

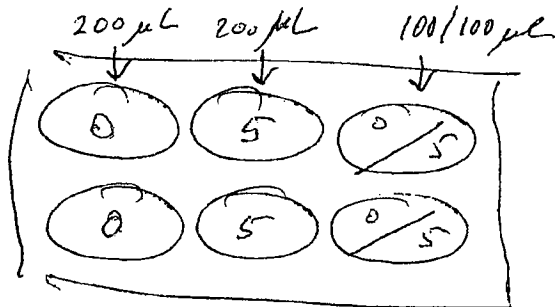
Time: 10:15

Trypsinization + Harvesting

0.5 mL Trypsin + 1 mL D-medium  
Total: 1.5 mL

Plating:

Time: 11:00



$$0.4 \times 10^6 \text{ cells}$$

To plate:  $\approx 50\%$  confluency, i.e.  $\approx 0.74 \times 10^6$  cells?

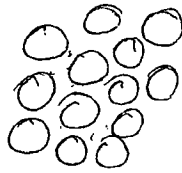
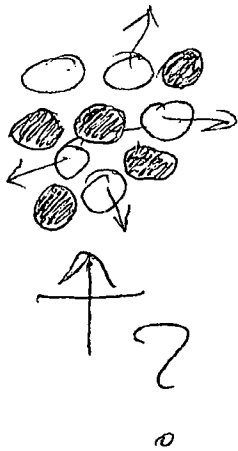
$$1.5 \text{ mL} - 3 \times 10^6 \text{ cells}$$
$$x \text{ mL} - 0.74 \times 10^6 \text{ cells}$$

$$x = 0.37 \text{ mL} \quad \left[ \begin{array}{l} 2 \text{ AGGREG} \\ \text{COEFFICIENT} \end{array} \right]$$

$$0.19 \text{ mL}$$

OR  $\approx 200 \mu\text{L}$

TO BE CONT. ↓



$$\begin{array}{r} 8.56 \\ 4.81 \\ \hline 14.37 \\ \hline 7.18 \end{array}$$

TIME : 9:00

$$\left. \begin{array}{l} 1436 \\ 1410 \\ 1453 \end{array} \right\} 1433$$

$$71 \times 10^5 \text{ cells/mL} + 0.5 \text{ mL} \rightarrow 8.56 \times 10^5 \text{ cells}$$

56g

$$\left. \begin{array}{l} 719 \\ 812 \\ 824 \end{array} \right\} 785$$

$$\left. \begin{array}{l} 822 \\ 885 \\ 749 \end{array} \right\} 819$$

$$802 \times 400 = 3.21 \times 10^5 \text{ cells/mL} + 0.5 \text{ mL} \rightarrow 4.81 \times 10^5 \text{ cells}$$

06g/56g

$$\left. \begin{array}{l} 1258 \\ 1207 \\ 1242 \end{array} \right\} 1236$$

$$\left. \begin{array}{l} 1345 \\ 1307 \\ 1327 \end{array} \right\} 1326$$

$$1281 \times 400 = 5.12 \times 10^5 \text{ cells/mL} + 0.5 \text{ mL} \rightarrow 7.68 \times 10^5 \text{ cells}$$

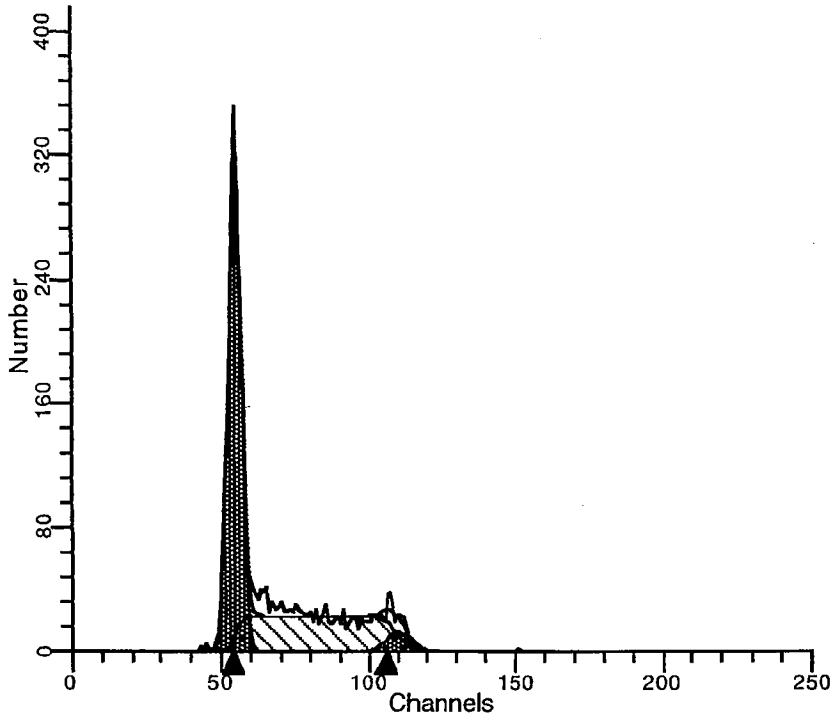


06g + 56g (Mixture)

$$\left. \begin{array}{l} 856 \\ 936 \\ 844 \end{array} \right\} 879$$

$$\left. \begin{array}{l} 1089 \\ 1109 \\ 1065 \end{array} \right\} 1088$$

$$984 \times 400 = 3.94 \times 10^5 \text{ cells/mL} + 0.5 \text{ mL} \rightarrow 5.91 \times 10^5 \text{ cells}$$



Date acquired: 15-May-2  
 File: 051902 .001  
 Source: ~~WBs OGY~~  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

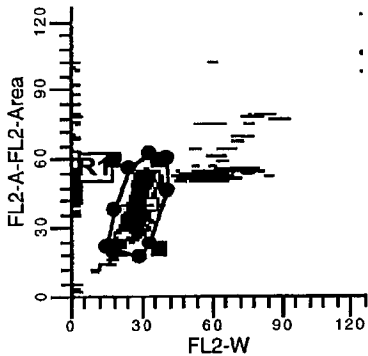
DIPLOID: 100.00 %  
 Dip G0-G1: 54.08 % at 54.59  
 Dip G2-M: 4.31 % at 109.53  
 Dip S: 41.61 % G2/G1: 2.01  
 Dip %CV: 3.81

Total S-Phase: 41.61 %

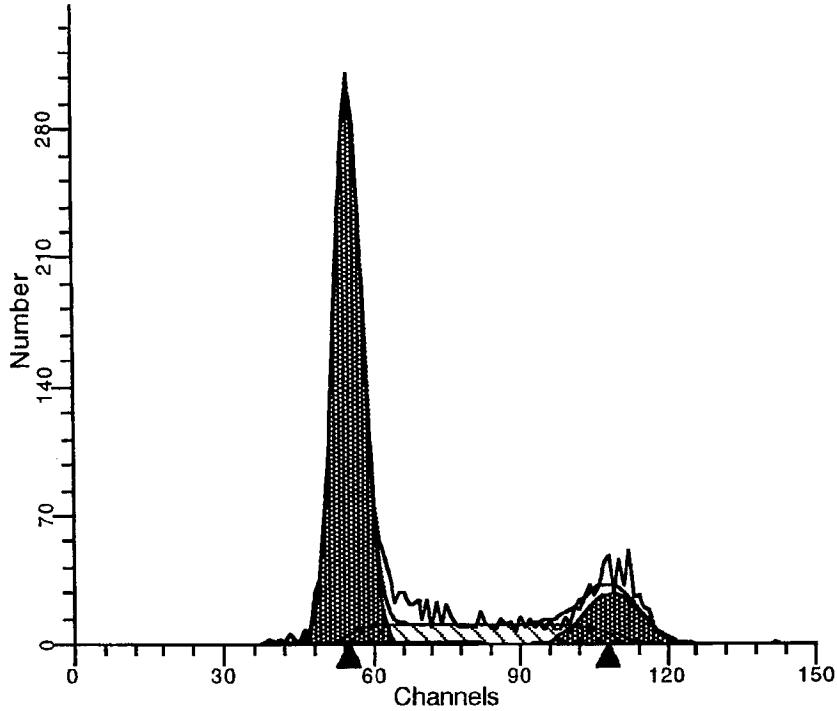
Extra Pop: %  
 Debris: 1.32 %  
 Aggregates: 0.00 %  
 Modeled Events: 3116  
 RCS: 0.470

Diploid B.A.D.: 0.70 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 15-May-2  
 File: 051902\_002  
 Source: WBs 5Gy  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

DIPLOID: 100.00 %  
 Dip G0-G1: 68.12 % at 55.00  
 Dip G2-M: 12.64 % at 108.65  
 Dip S: 19.24 % G2/G1: 1.98  
 Dip %CV: 5.20

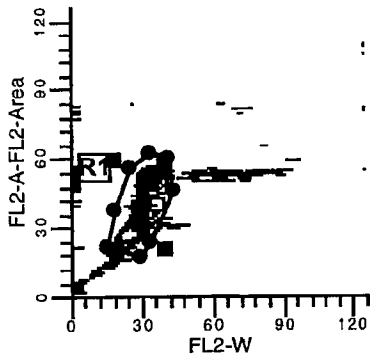
*delay*

Total S-Phase: 19.24 %

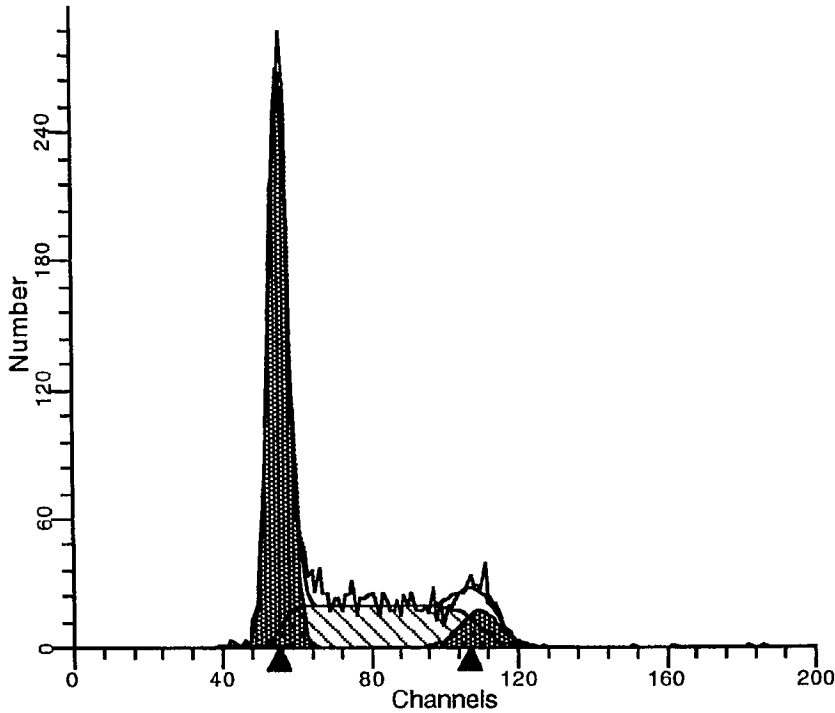
Extra Pop: %  
 Debris: 2.08 %  
 Aggregates: 0.00 %  
 Modeled Events: 3206  
 RCS: 1.342

Diploid B.A.D.: 1.29 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 15-May-2  
 File: 051902\_003  
 Source: WBs 0/5Gy  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

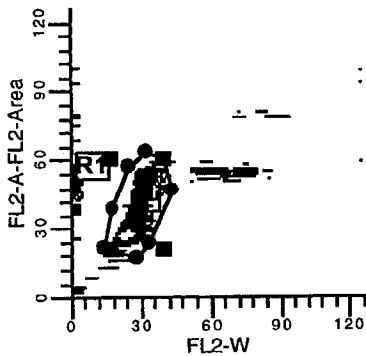
DIPLOID: 100.00 %  
 Dip G0-G1: 57.89 % at 55.38  
 Dip G2-M: 7.34 % at 109.63  
 Dip S: 34.78 % G2/G1: 1.98  
 Dip %CV: 4.88

Total S-Phase: 34.78 %

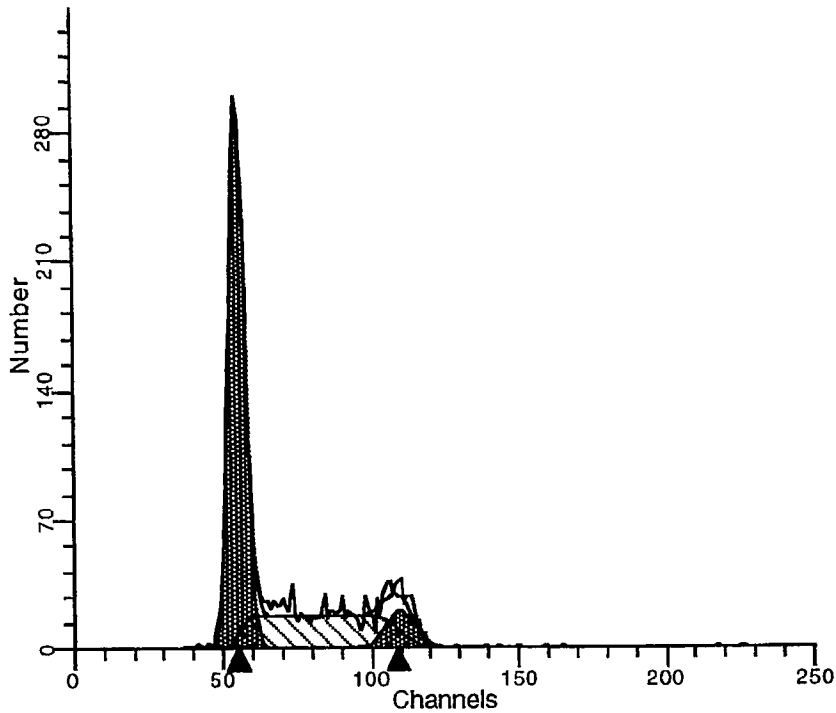
Extra Pop: %  
 Debris: 0.87 %  
 Aggregates: 0.00 %  
 Modeled Events: 3129  
 RCS: 0.590

Diploid B.A.D.: 0.13 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 15-May-2  
 File: 051902\_004  
 Source: WBs 0Gy+5Gy MIXTURE  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

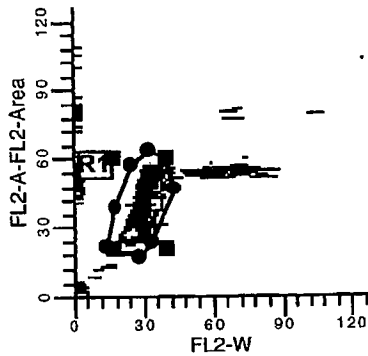
DIPLOID: 100.00 %  
 Dip G0-G1: 60.24 % at 55.18  
 Dip G2-M: 8.17 % at 109.81  
 Dip S: 31.59 % G2/G1: 1.99  
 Dip %CV: 4.60

Total S-Phase: 31.59 %

Extra Pop: %  
 Debris: 1.17 %  
 Aggregates: 0.00 %  
 Modeled Events: 3150  
 RCS: 0.591

Diploid B.A.D.: 0.22 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFRILT V2.0(PMac)

MAY 16, 2002

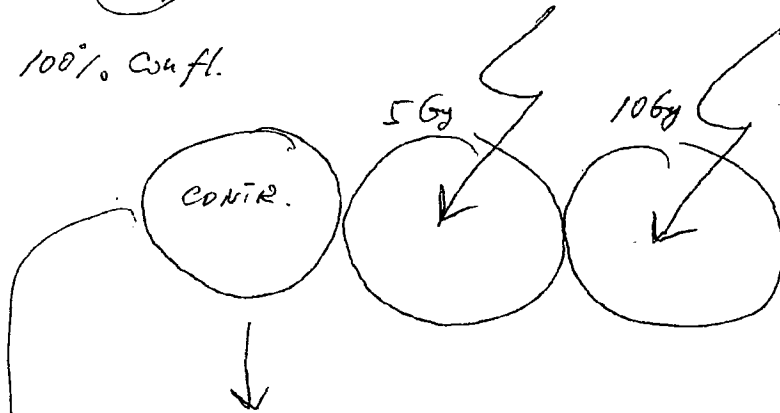
BYSTANDER EFFECTS ( $\gamma$ -IRRADIATION)

WB (39p)

$^{137}\text{Cs}$

100% confl.

Time: 8:15



One source: 345.59 Rad/min

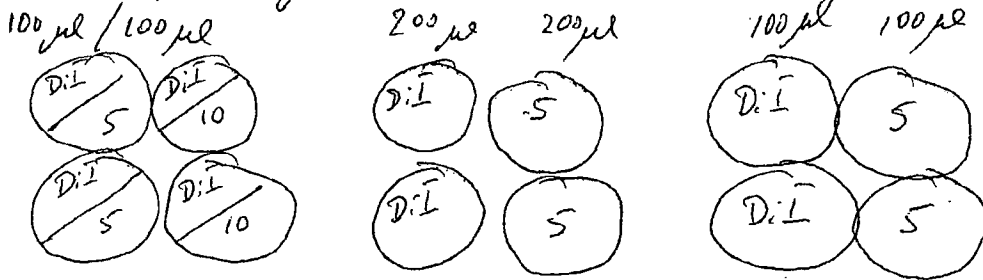
5 Gy  $\rightarrow$  1.45 min  
10 Gy  $\rightarrow$  2.89 min

DiI staining ( $\approx 40\text{min}$ )

Washing with PBS (2x)

Trypsinization + Harvesting (0.5 mL Trypsin + 1 mL D-medium)

Plating:  $\approx 10:00$



$\rightarrow$  Counter Counts:

$\left. \begin{matrix} 5968 \\ 5908 \\ 5934 \end{matrix} \right\} 5937 \times 400 \approx 2.4 \times 10^6 \text{ cells/ml}$

To plate:  $200\mu\text{l} - 0.48 \times 10^6 \text{ cells}$  OR  
 $100\mu\text{l} - 0.24 \times 10^6 \text{ cells}$



MAY 17, 2002.

FIXATION IN FA (2.12%). 1.5 mL aliquots  
TIME: 8:00

COULTER COUNTS

0 Gy / 5 Gy }  
1450 }  
1439 } 1464  
1504 }

0 Gy / 5 Gy }  
1364 }  
1336 } 1337  
1312 }

$1401 \times 400 = 5.6 \times 10^5 \text{ cells/mL}$   
 $+ 0.5 \text{ mL} \rightarrow 8.4 \times 10^5 \text{ cells}$

0 Gy / 10 Gy }  
1691 }  
1673 } 1696  
1723 }

0 Gy / 10 Gy }  
1605 }  
1632 } 1595  
1549 }

$1645 \times 400 = 6.58 \times 10^5 \text{ cells/mL}$   
 $+ 0.5 \text{ mL} \rightarrow 9.87 \times 10^5 \text{ cells}$

~~0 Gy~~ (200  $\mu$ L) }  
2149 }  
2169 } 2132  
2077 }

0 Gy (200  $\mu$ L) }  
1818 }  
1784 } 1782  
1746 }

$1957 \times 400 = 7.83 \times 10^5 \text{ cells/mL}$   
 $+ 0.5 \text{ mL} \rightarrow 11.74 \times 10^5 \text{ cells}$

$R \left( \frac{0 \text{ Gy}}{5 \text{ Gy}} \right) = 1.52$

5 Gy (200  $\mu$ L) }  
1173 }  
1122 } 1164  
1197 }

5 Gy (200  $\mu$ L) }  
~~1484~~ }  
1436 }  
1369 } 1412  
1430 }

$1288 \times 400 = 5.15 \times 10^5 \text{ cells/mL}$   
 $+ 0.5 \text{ mL} \rightarrow 7.73 \times 10^5 \text{ cells}$

0 Gy (100  $\mu$ L)

1063 }  
1083 } 1058  
1028 }

0 Gy (100  $\mu$ L)

1100 }  
1084 } 1110  
1146 }

$1084 \times 400 = 4.34 \times 10^5 \text{ cells/mL}$   
 $+ 0.5 \text{ mL} \rightarrow 6.51 \times 10^5 \text{ cells}$

$R\left(\frac{0 \text{ Gy}}{5 \text{ Gy}}\right) = 1.56$

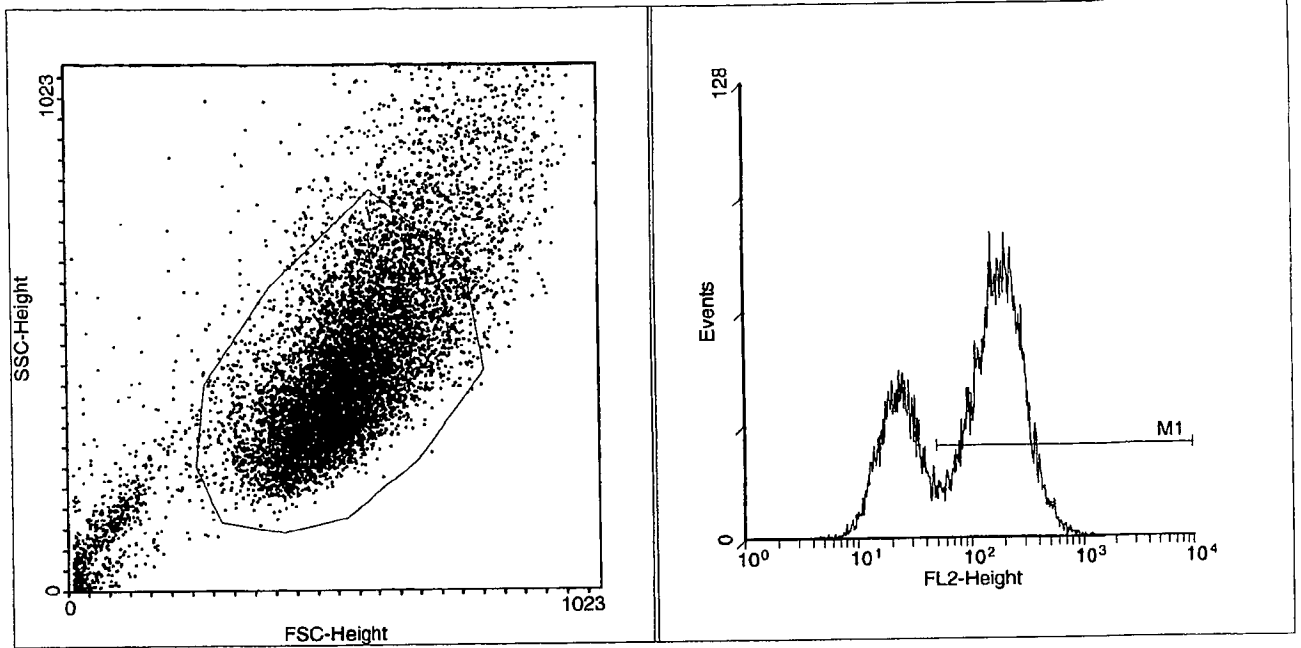
5 Gy (100  $\mu$ L)

696 }  
706 } 710  
728 }

5 Gy (100  $\mu$ L)

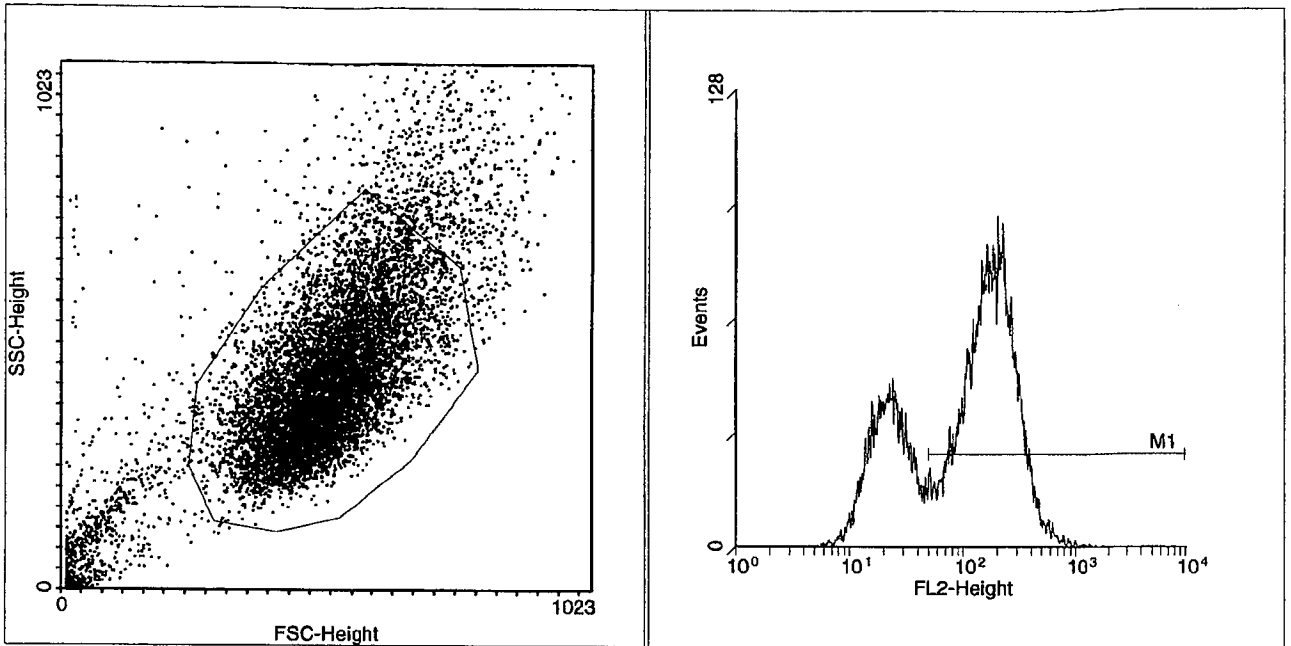
680 }  
661 } 683  
707 }

$897 \times 400 = 2.79 \times 10^5 \text{ cells/mL}$   
 $+ 0.5 \text{ mL} \rightarrow 4.18 \times 10^5 \text{ cells}$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri May 17 15:03:35 2002  
 Gates: R1  
 Project: Experiment:  
 File: 051202 Sample: WB 0Gy(DiI)/5Gy  
 Date: 17-May-2 Parameters: 1  
 Total Events 20000 Gated Events 15813 79.07%  
 System: Log Parameter Means: Geometric  

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	15813	79.07	100.00	92.68	23.65	94,153.993
	1	434,1023	10949	54.75	69.24	174.49	10.31	94,153.993



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri May 17 14:58:41 2002

Gates: R1

Project: Experiment:

File: 051202 Sample: WB 0Gy (DiI) / 5Gy

Date: 17-May-2 Parameters: 1

Total Events 20000 Gated Events 16639 83.19%

System: Log Parameter Means: Geometric

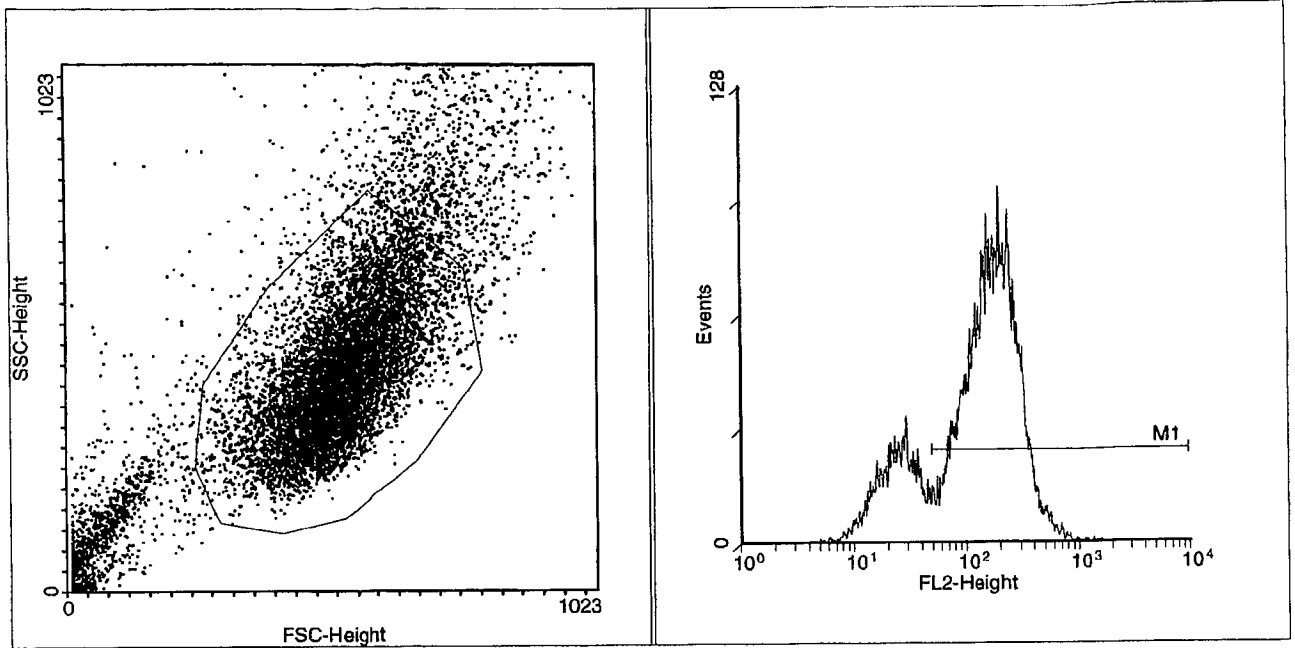
Param name	M	Low, High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0, 1023	16639	83.19	100.00	92.73	23.67	100,205.353
	1	434, 1023	11633	58.17	69.91	172.55	10.46	100,205.353

+ 69.24

139.15

69.58

Ratio:  $\frac{69.6}{30.4} = 2.29$  OR  $2.3$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri May 17 15:05:34 2002

Gates: R1

Project: Experiment:

File: 051202 Sample: WB 0Gy(DiI)/10Gy

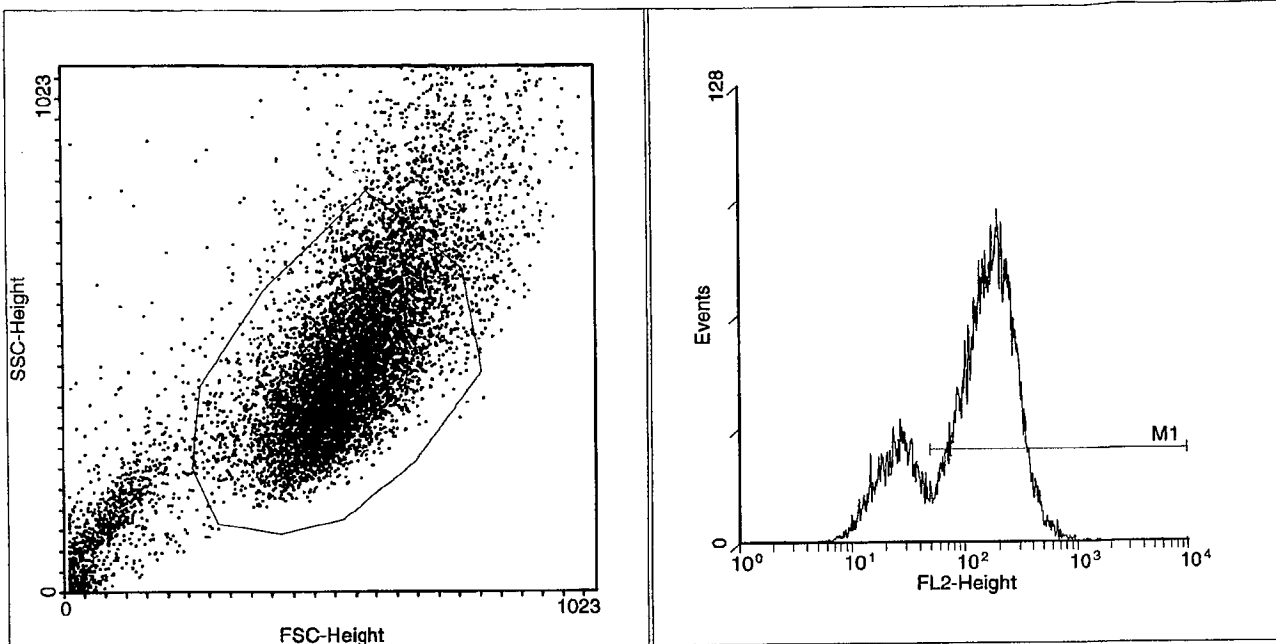
Date: 17-May-2 Parameters: 1

Total Events 20000 Gated Events 15435 77.18%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	15435	77.18	100.00	111.75	20.38	107,239.28
	1	434,1023	12159	60.80	78.78	170.68	10.28	107,239.28

*Ratio:*



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri May 17 15:07:02 2002

Gates: R1

Project: Experiment:

File: 051202 Sample: (WB 0Gy (DiI) / 10Gy)

Date: 17-May-2 Parameters: 1

Total Events 20000 Gated Events 15333 (76.67%)

System: Log Parameter Means: Geometric

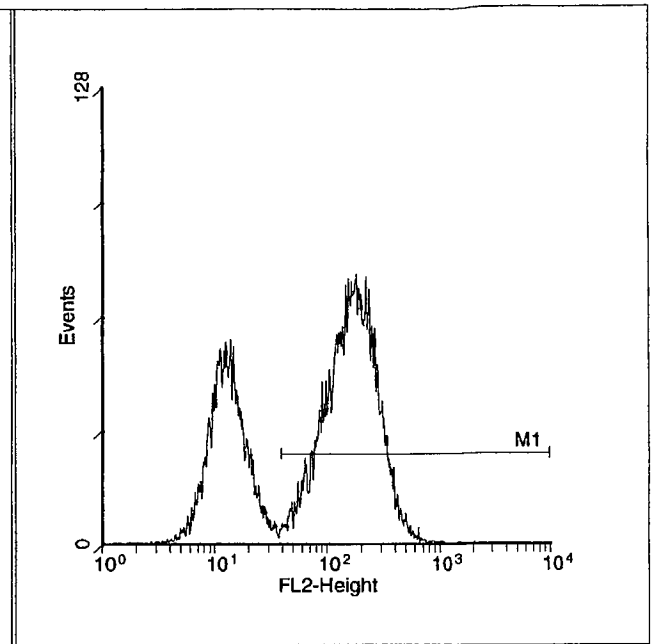
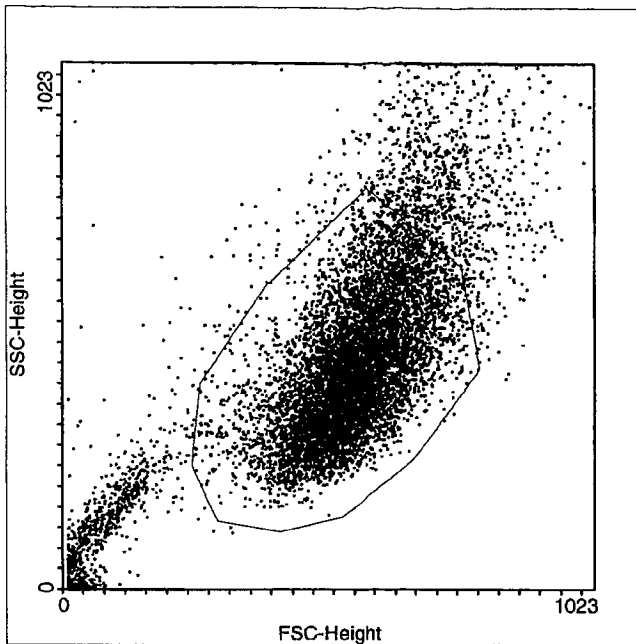
Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	15333	76.67	100.00	108.16	20.60	100,198.096
	1	434,1023	11917	59.59	(77.72)	167.79	10.32	100,198.096

+ 78.78

156.5

78.25

Ratio:  $\frac{78.3}{21.7} = 3.6$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri May 17 15:10:19 2002

Gates: R1

Project: Experiment:

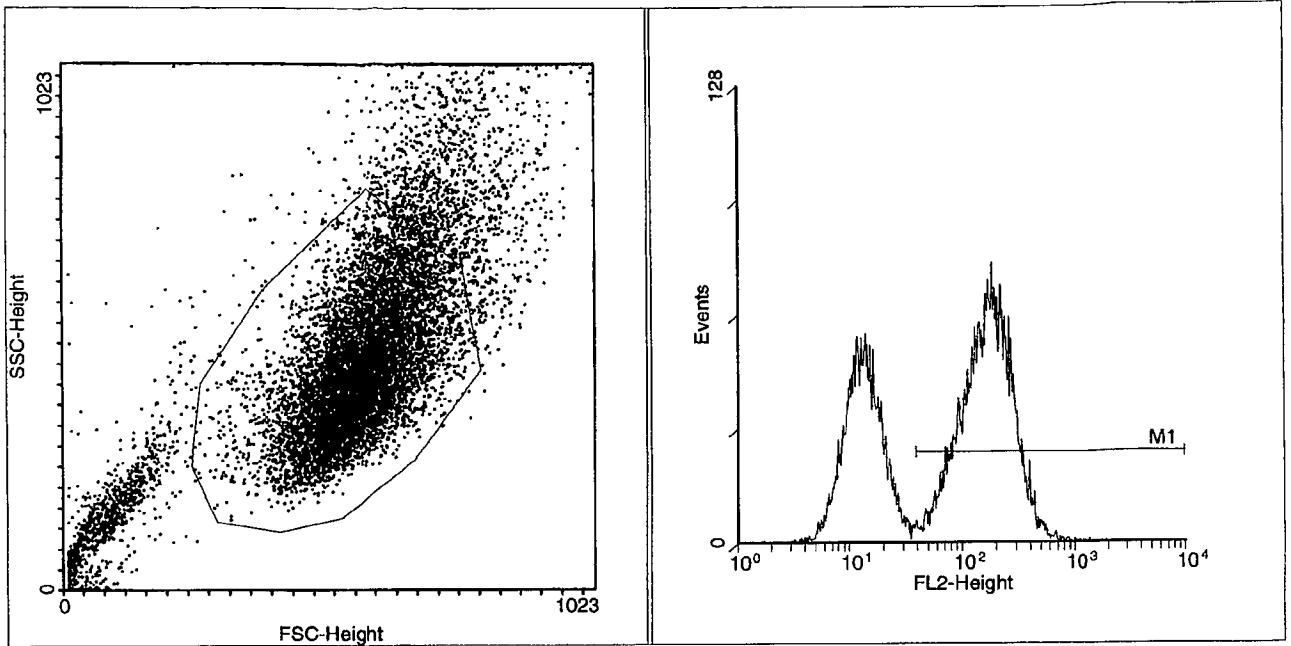
File: 051202 Sample: WB 0Gy+5Gy/100microL

Date: 17-May-2 Parameters: 1

Total Events 20000 Gated Events 15788 78.94%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	15788	78.94	100.00	67.62	30.81	83,218.697
	1	407,1023	10348	51.74	65.54	161.46	10.54	83,218.697



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri May 17 15:14:23 2002

Gates: R1

Project: Experiment:

File: 051202 Sample: WB 0Gy+5Gy/100microl

Date: 17-May-2 Parameters: 1

Total Events 20000 Gated Events 15381 76.90%

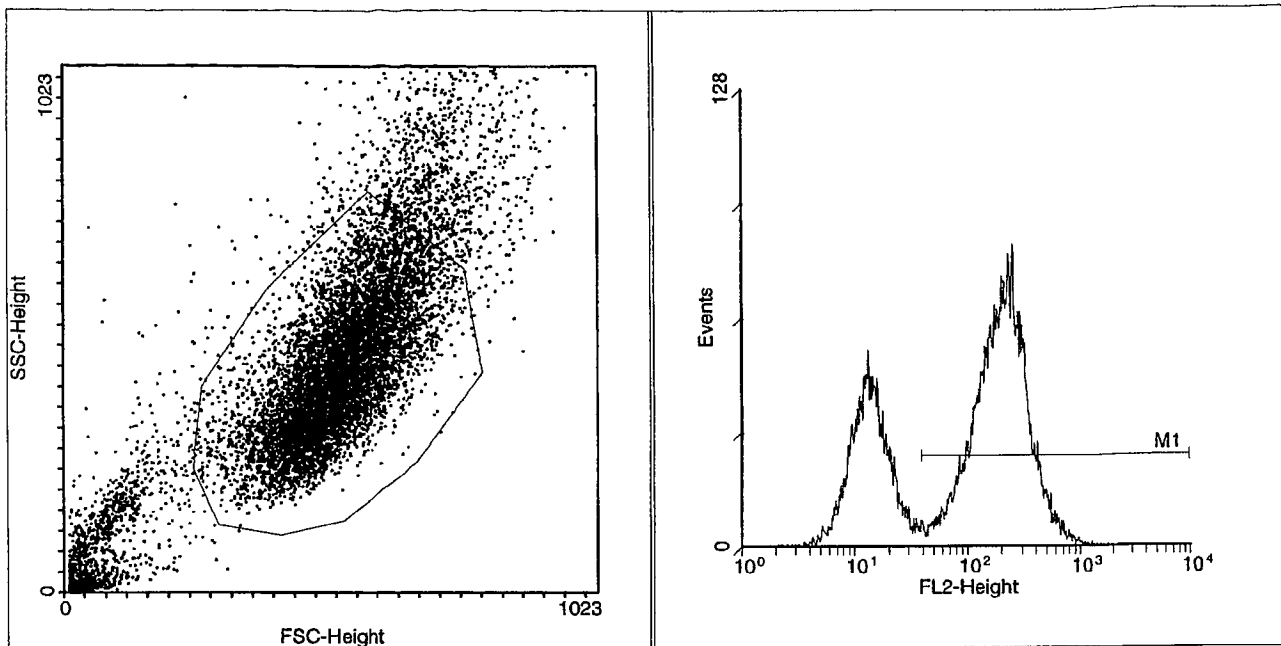
System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	15381	76.91	100.00	63.53	31.53	86,182.692
	1	407,1023	9603	48.02	<u>62.43</u>	163.17	10.40	86,182.692

$$\begin{array}{r}
 + 65.54 \\
 \hline
 127.97 \quad | \quad 2 \\
 \hline
 63.99
 \end{array}$$

$$R = \frac{0Gy : 64.0}{5Gy : 36.0} = \frac{1.77}{1.8}$$





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri May 17 15:18:00 2002

Gates: R1

Project: Experiment:

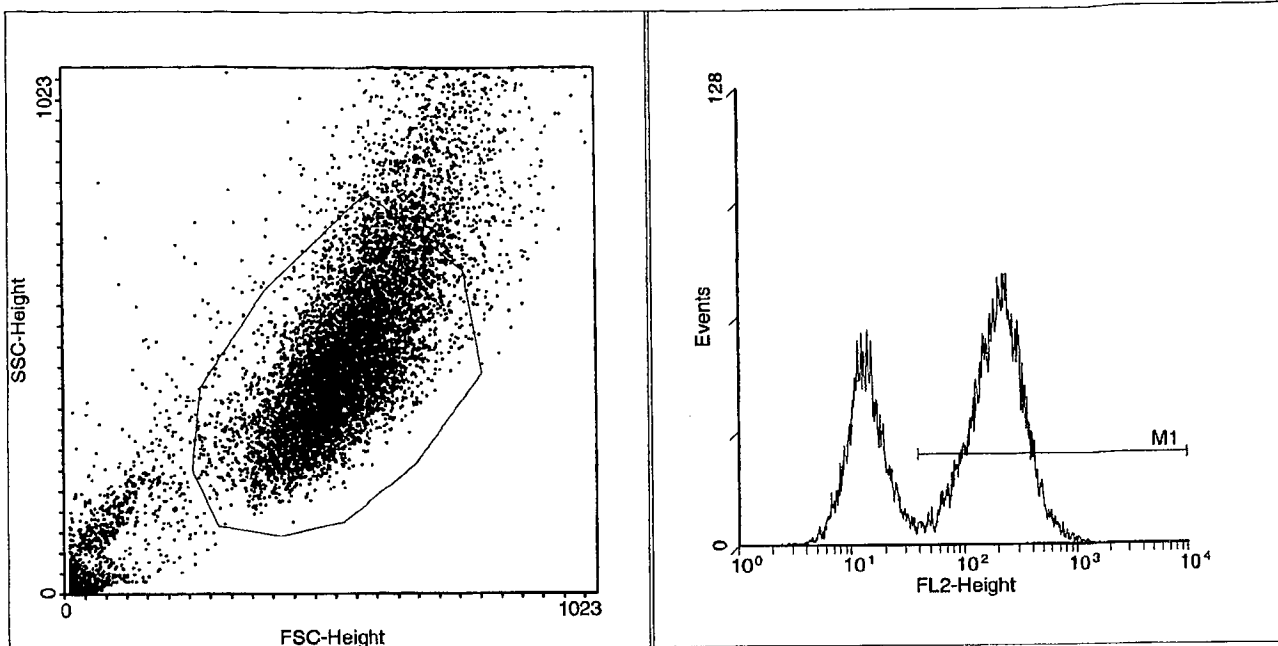
File: 051202 Sample: WR\_0Gy+5Gy/200microL

Date: 17-May-2 Parameters: 1

Total Events 20000 Gated Events 15279 76.39%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	15279	76.40	<u>100.00</u>	81.90	30.72	94,228.757
	1	407,1023	10317	51.59	<u>67.52</u>	194.74	10.72	94,228.757



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri May 17 15:18:37 2002  
 Gates: R1

Project: Experiment:  
 File: 051202 Sample: WB 0Gy+5Gy/200microL  
 Date: 17-May-2 Parameters: I  
 Total Events 20000 Gated Events 15192 (75.96%)  
 System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	15192	75.96	100.00	76.79	31.50	87,237.137
	1	407,1023	9954	49.77	65.52	191.96	10.94	87,237.137

$$+66.52$$

$$\frac{133.04}{2} = 66.52$$

$$K = \frac{0Gy: 66.5}{5Gy: 33.5} = 1.99 \text{ OR } \boxed{2.0}$$

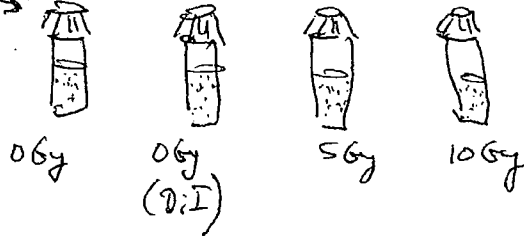
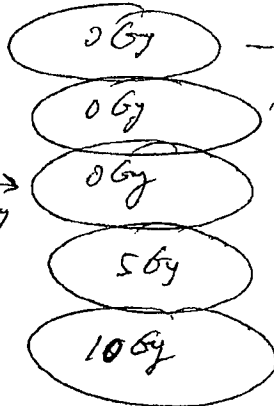
MAY 19, 2002

# BYSTANDER EFFECTS (f-IRRADIATION)

WB (40p)

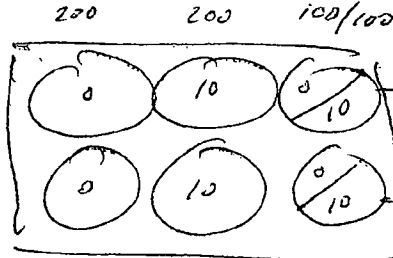
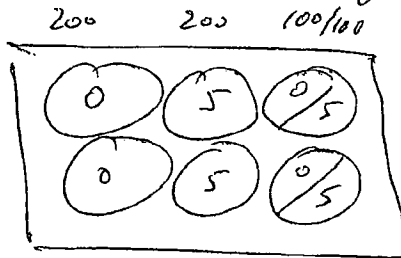
IRRADIATION TIME: 11:10  
(One source: 345.59 Rad/min)

For DiI staining  
(40 min)



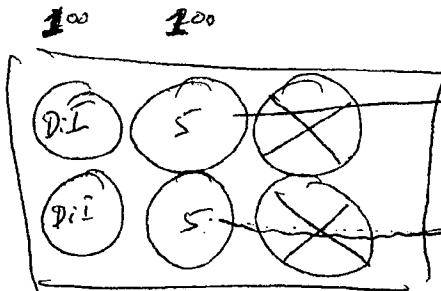
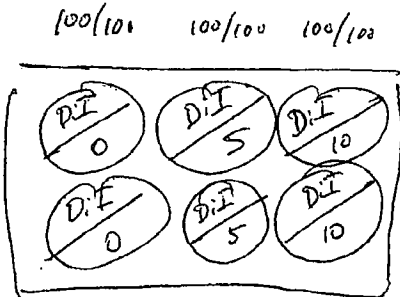
PLATING TIME: 12:30

~~FOR DNA~~ FOR DNA



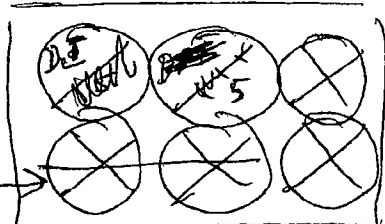
to be combined  
before FCM

FOR PROLIFERATIVE ACTIVITY

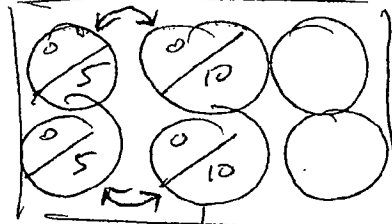


to be combined  
before FCM

200    200



100/100    100/100



FOR DiI staining prior  
to FACS

Amount of aliquot in tube was not enough (5 Gy) (-100  $\mu$ L).

COLIE COUNTS :

$$\left. \begin{array}{l} 4939 \\ 4827 \\ 4904 \end{array} \right\} 4890 \times 400 \approx 2.0 \times 10^6 \text{ cells/ml}$$

$$\begin{array}{l} \text{To plate: } 100 \mu\text{l} : 0.2 \times 10^6 \text{ cells} \\ \quad \quad \quad 200 \mu\text{l} : 0.4 \times 10^6 \text{ cells} \end{array}$$

COULTER COUNTS:

~~D.I (0.6g)~~

0 Gy - oh. 1705 }  
 1748 }  $1722 \times 400 = 6.89 \times 10^5 \text{ cells/mL}$   
 1713 }  $+0.5 \text{ mL} \rightarrow 10.34 \times 10^5 \text{ cells}$

0 Gy (100  $\mu$ L) 1760 }  
 1795 }  $1782 \times 400 = 7.13 \times 10^5 \text{ cells/mL}$   
 1790 }  $+0.5 \text{ mL} \rightarrow 10.7 \times 10^5 \text{ cells}$   
 $R(\frac{0.6g}{5Gy}) = 1.49$

5 Gy (100  $\mu$ L) 1241 }  
 1229 }  $1195 \times 400 = 4.78 \times 10^5 \text{ cells/mL}$   
 1116 }  $+0.5 \rightarrow 7.17 \times 10^5 \text{ cells}$

0 Gy (200  $\mu$ L) 1959 }  
 1918 }  $1974 \times 400 = 7.89 \times 10^5 \text{ cells/mL}$   
 2044 }  $+0.5 \rightarrow 11.84 \times 10^5 \text{ cells}$   
 $R(\frac{0.6g}{5Gy}) = 1.83$

5 Gy (200  $\mu$ L) 1064 }  
 1104 }  $1080 \times 400 = 4.32 \times 10^5 \text{ cells/mL}$   
 1072 }  $+0.5 \rightarrow 6.48 \times 10^5 \text{ cells}$

0 Gy / 0 Gy 1729 }  
 1752 }  $1706 \times 400 = 6.83 \times 10^5 \text{ cells/mL}$   
 1638 }  $+0.5 \rightarrow 10.25 \times 10^5 \text{ cells}$   
 $\text{Ave. } 10.43 \times 10^5 \text{ cells}$

0 Gy / 0 Gy 1776 }  
 1732 }  $1767 \times 400 = 7.07 \times 10^5 \text{ cells/mL}$   
 1792 }  $+0.5 \rightarrow 10.61 \times 10^5 \text{ cells}$

0 Gy / 5 Gy 1429 }  
 1317 }  $1387 \times 400 = 5.55 \times 10^5 \text{ cells/mL}$   
 1415 }  $+0.5 \rightarrow 8.33 \times 10^5 \text{ cells/mL}$   
 $\text{Ave. } 9.23 \times 10^5 \text{ cells}$

0 Gy / 5 Gy 1736 }  
 1711 }  $1687 \times 400 = 6.75 \times 10^5 \text{ cells/mL}$   
 1613 }  $+0.5 \rightarrow 10.13 \times 10^5 \text{ cells/mL}$

0.6g / 10.6g

$$\left. \begin{array}{l} 1467 \\ 1444 \\ 1484 \end{array} \right\} 1465 \times 400 = 5.86 \times 10^5 \text{ cells/mL}$$

$$+ 0.5 \text{ mL} \rightarrow 8.79 \times 10^5 \text{ cells}$$

$$\text{Ave. } 8.97 \times 10^5 \text{ cells}$$

0.6g / 10.6g

$$\left. \begin{array}{l} 1465 \\ 1528 \\ 1578 \end{array} \right\} 1523 \times 400 = 6.09 \times 10^5 \text{ cells/mL}$$

$$+ 0.5 \text{ mL} \rightarrow 9.14 \times 10^5 \text{ cells}$$

HEMOCYTOMETER COUNTS:

MAY 22, 2002

0 Gy (D.I.) / (64) / 0 Gy  
 214 } ~~242~~ <sup>483</sup> = ~~242~~ × 10<sup>3</sup> cells/mL  
 269 } ~~OR 242 × 10<sup>3</sup> cells/mL~~  
 + 0.5 mL → ~~263 × 10<sup>3</sup> cells~~ 1.5 mL aliquot  
 after FA-fixation  
 725  
 Actually 0.2 mL aliquot contains ~~263~~ × 10<sup>3</sup> cells  
 OR 1.5 mL ~~5.442~~ × 10<sup>6</sup> cells (0.5 cm dish)  
 → Plated ~~263 × 10<sup>3</sup> cells~~ (725 × 10<sup>3</sup> cells)

0 Gy (100 μL) 275 } ~~501~~ <sup>501</sup> = ~~501~~ × 10<sup>3</sup> cells/mL  
 226 } + 0.5 mL → ~~377 × 10<sup>3</sup> cells~~ 752 × 10<sup>3</sup> cells

5 Gy (100 μL) 132 } 284 × 10<sup>3</sup> cells/mL  
 152 } + 0.5 mL → 426 × 10<sup>3</sup> cells

0 Gy (200 μL) 313 } 598 × 10<sup>3</sup> cells/mL  
 285 } + 0.5 mL → 897 × 10<sup>3</sup> cells

5 Gy (200 μL) 259 - 518 × 10<sup>3</sup> cells/mL  
 + 0.5 → 777 × 10<sup>3</sup> cells

0 Gy / 0 Gy 317 } 567 × 10<sup>3</sup> cells/mL  
 250 } + 0.5 mL → 851 × 10<sup>3</sup> cells  
 Ave. 710 × 10<sup>3</sup> cells

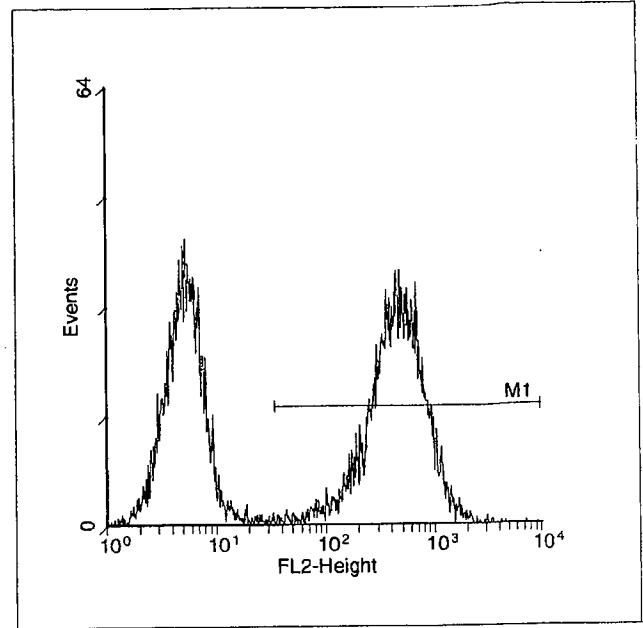
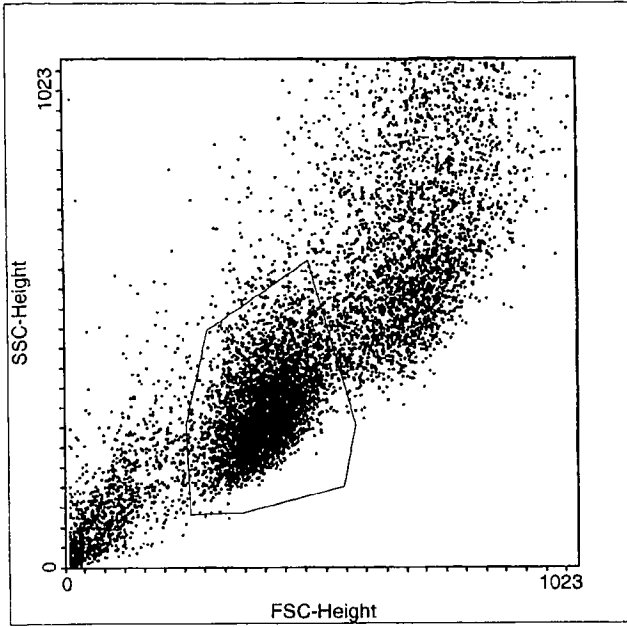
0 Gy / 0 Gy 223 } 459 × 10<sup>3</sup> cells/mL  
 236 } + 0.5 mL → 689 × 10<sup>3</sup> cells

0 Gy / 5 Gy 217 } 434 × 10<sup>3</sup> cells/mL  
 217 } + 0.5 mL → 651 × 10<sup>3</sup> cells  
 Ave. 677 × 10<sup>3</sup> cells

0 Gy / 5 Gy 232 } 468 × 10<sup>3</sup> cells/mL  
 236 } + 0.5 mL → 702 × 10<sup>3</sup> cells

0 Gy / 10 Gy 134 } ~~140~~ <sup>140</sup> } 280 × 10<sup>3</sup> cells/mL  
 146 } + 0.5 → 420 × 10<sup>3</sup> cells  
 Ave. 474 × 10<sup>3</sup> cells

0 Gy / 10 Gy 180 } 352 × 10<sup>3</sup> cells/mL  
 172 } + 0.5 → 528 × 10<sup>3</sup> cells



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 20 14:39:31 2002

Gates: R1

Project: Experiment:

File: 052002 Sample: 0Gy+0Gy(DiI) 0h

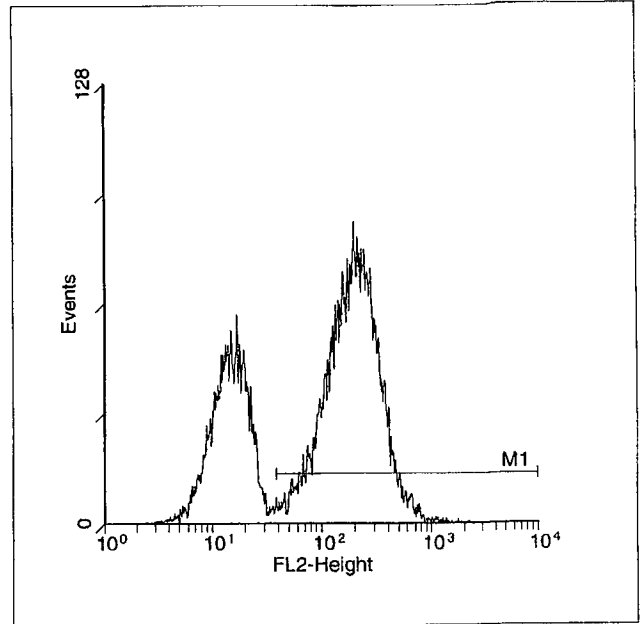
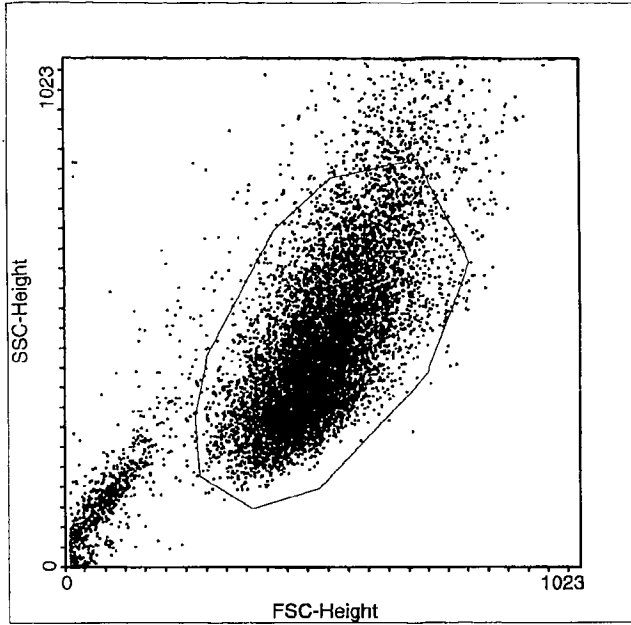
Date: 20-May-2 Parameters: 1

Total Events 20000 Gated Events 9064 45.32%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	9064	45.32	100.00	62.38	54.86	47,5.42469
	1	394,1023	5098	25.49	56.24	432.13	10.73	43,567.422





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 20 14:49:05 2002

Gates: R1

Project: Experiment:

File: 052002 Sample: 0Gy (DiI) +5Gy 100microL

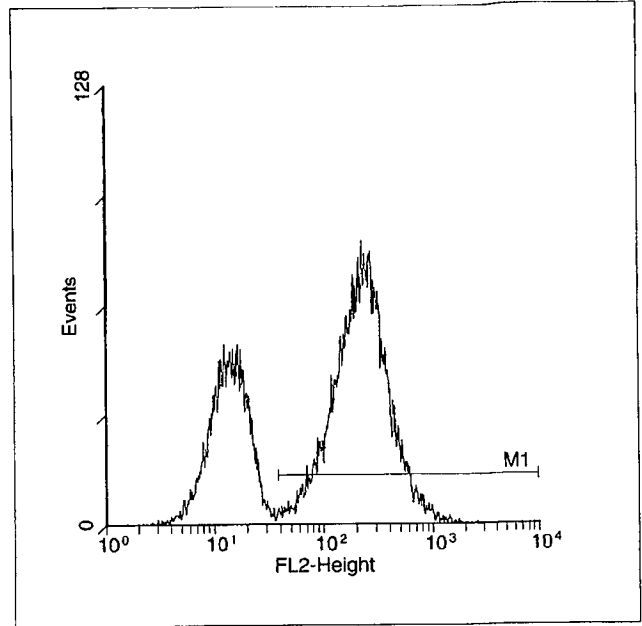
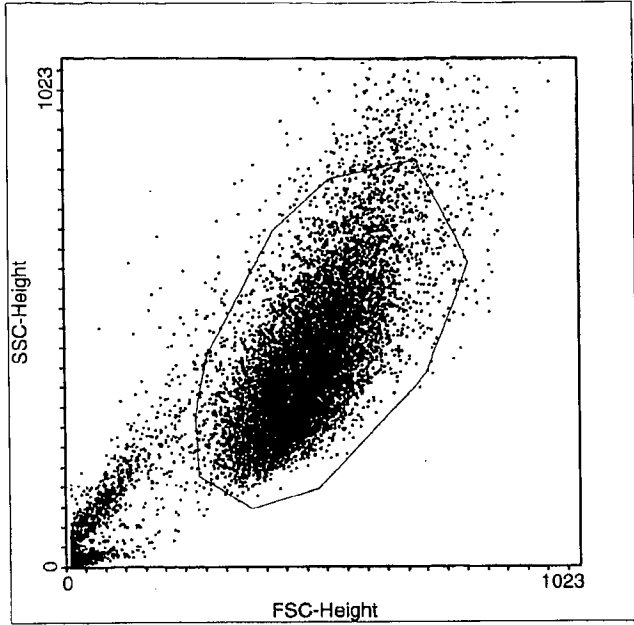
Date: 20-May-2 Parameters: 1

Total Events 20000 Gated Events 17105 85.53%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	17105	85.52	100.00	81.34	30.08	96,201.691
	1	406,1023	11473	57.37	67.07	190.67	10.85	96,201.691

$$\text{Ratio: } \frac{67.07}{32.93} = 2.0$$



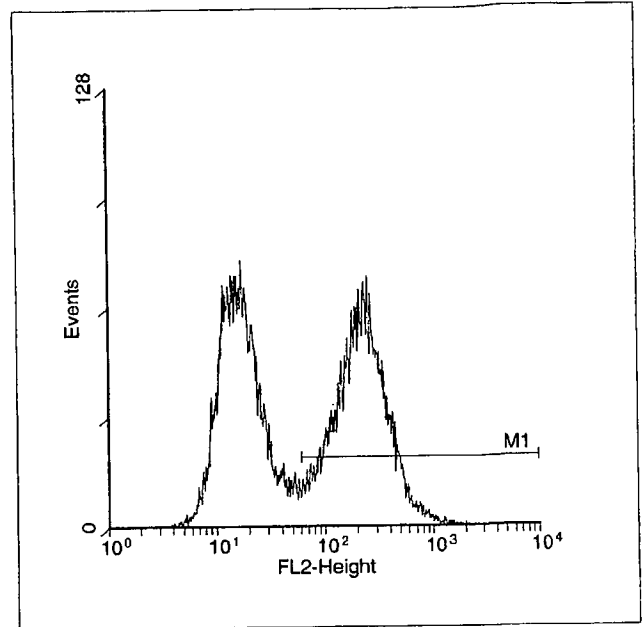
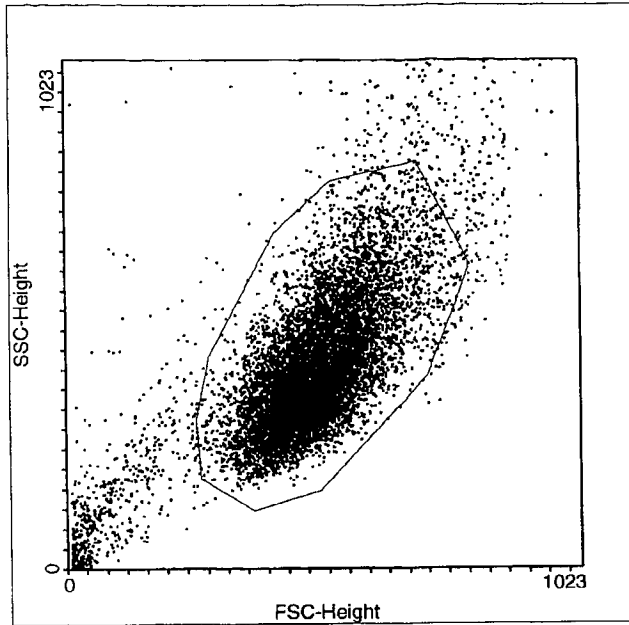
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 20 14:49:37 2002  
 Gates: R1

Project: Experiment:  
 File: 052002 Sample: 0Gy (DiI) + 5Gy 200microl  
 Date: 20-May-2 Parameters: 1

Total Events 20000 Gated Events 16746 83.73%  
 System: Log Parameter Means: Geometric

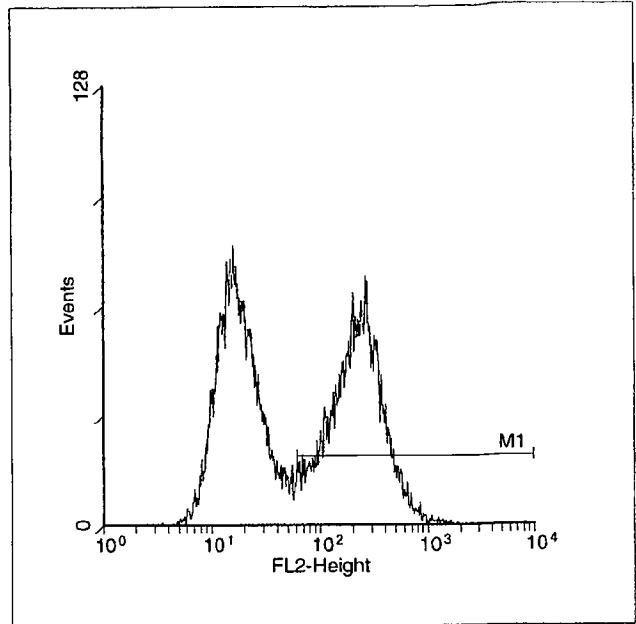
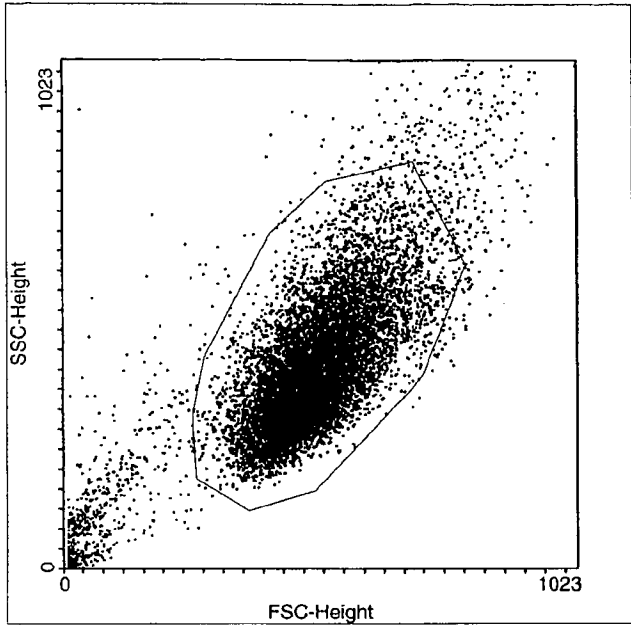
Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	16746	83.73	100.00	86.58	31.93	91,226.709
	1	406,1023	11185	55.93	<u>66.79</u>	218.88	11.16	91,226.709

Ratio:  $\frac{66.79}{33.21} = 2.0$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 20 14:50:23 2002  
 Gates: R1  
 Project: Experiment  
 File: 052002 Sample: 0Gy/0Gy (DII)  
 Date: 20-May-2 Parameters: 1  
 Total Events 20000 Gated Events 17958 89.79%  
 System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	17958	89.79	100.00	62.71	33.12	88,17.1544
	1	406,1023	9830	49.15	54.74	195.80	12.55	79,248.045



Multiple Document Interface for Flow Cytometry

WinMDI Version 2.8 - Windows 3.95/DOS 7.10

Mon May 20 14:55:23 2002

Gates: R1

Project: Experiment:

File: 052002 Sample: 0Gy/0Gy (Di-)

Date: 20-May-2 Parameters: 1

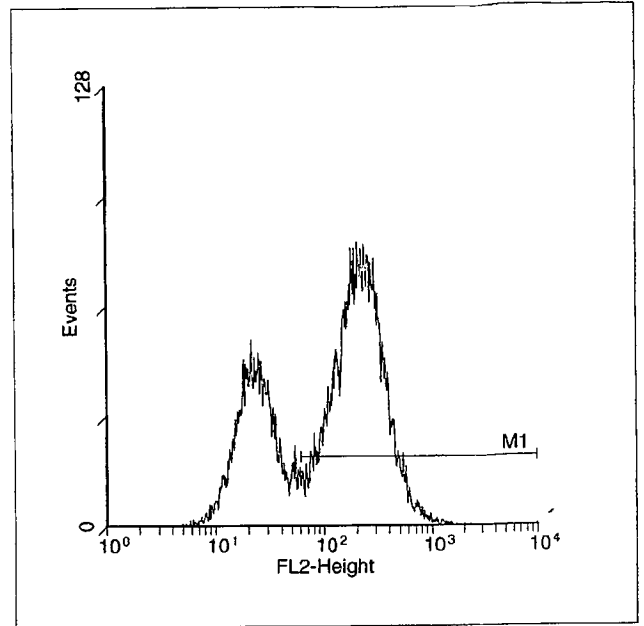
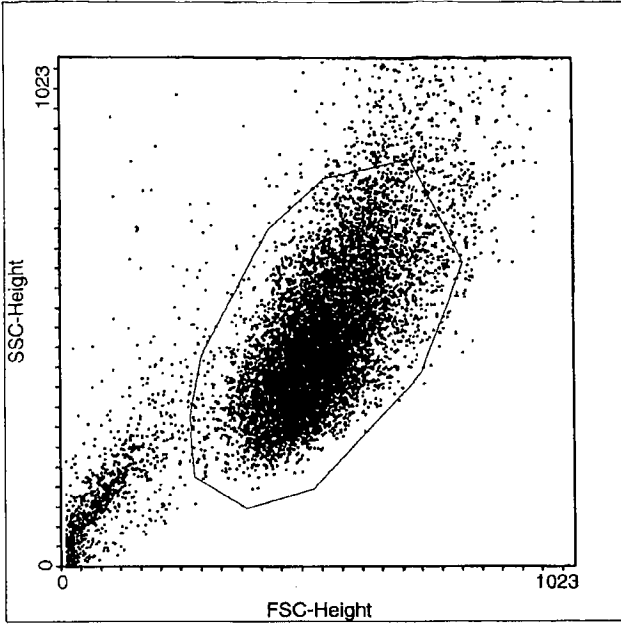
Total Events 20000 Gated Events 17982 89.91%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	17982	89.91	100.00	63.18	32.53	93,15.8204
	1	457,1023	9104	45.52	50.63	215.18	10.71	77,259.455

+ 54.74

105.37 | 2  
52.7



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 20 14:56:44 2002

Gates: R1

Project: Experiment:

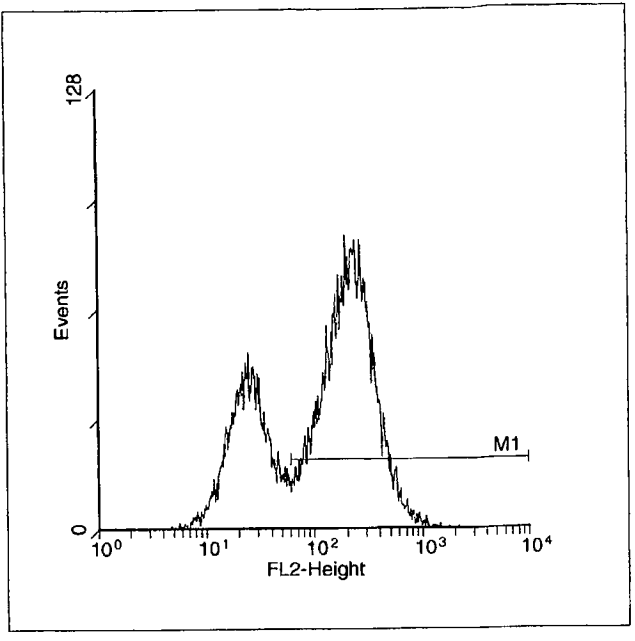
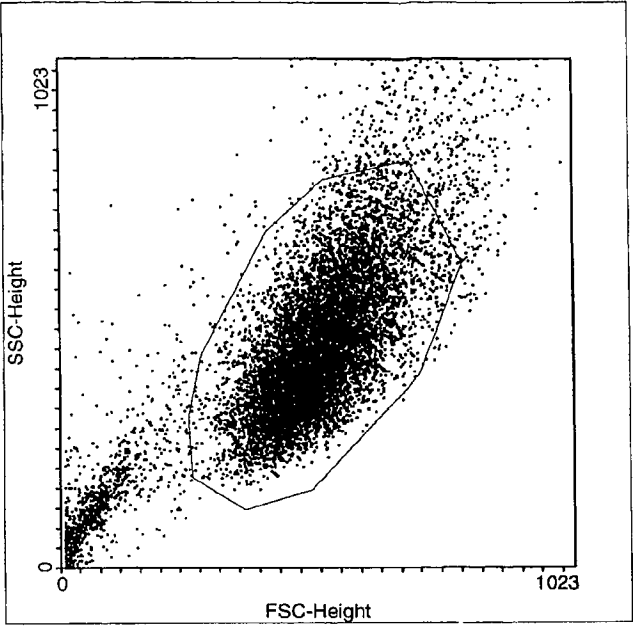
File: 052002 Sample: 5Gy/0Gy(DiI)

Date: 20-May-2 Parameters: 1

Total Events 20000 Gated Events 16784 83.92%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	16784	83.92	100.00	101.83	24.98	93,237.137
	1	457,1023	11224	56.12	66.87	210.88	10.15	93,237.137



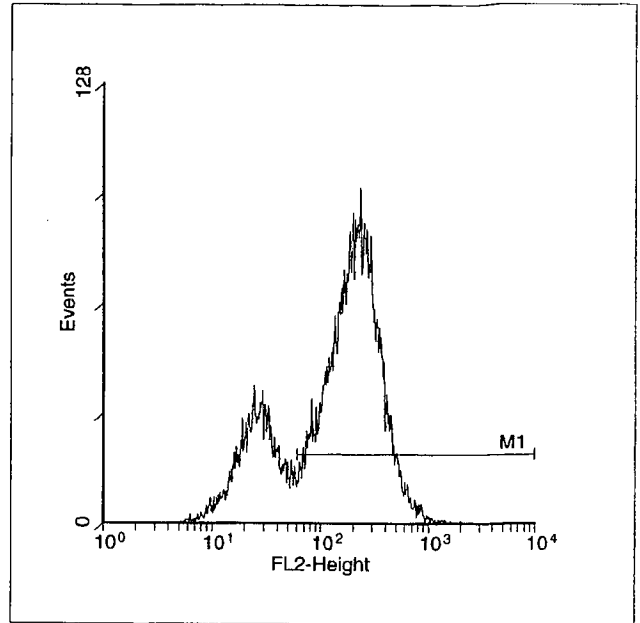
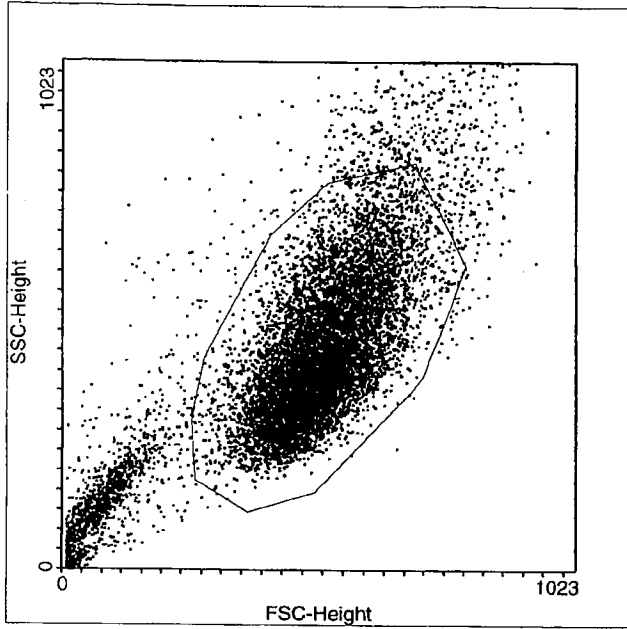
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 20 14:57:18 2002  
 Gates: R1

Project: Experiment:  
 File: 052002 Sample: 5Gy/0Gy(DIT)  
 Date: 20-May-2 Parameters: 1  
 Total Events 20000 Gated Events 16822 84.11%  
 System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	16822	84.11	100.00	105.28	24.33	95,196.322
	1	457,1023	11434	57.17	67.97	210.26	10.24	95,196.322

+66.87  
~~67.42~~  
 134.84 / 2 = 67.42

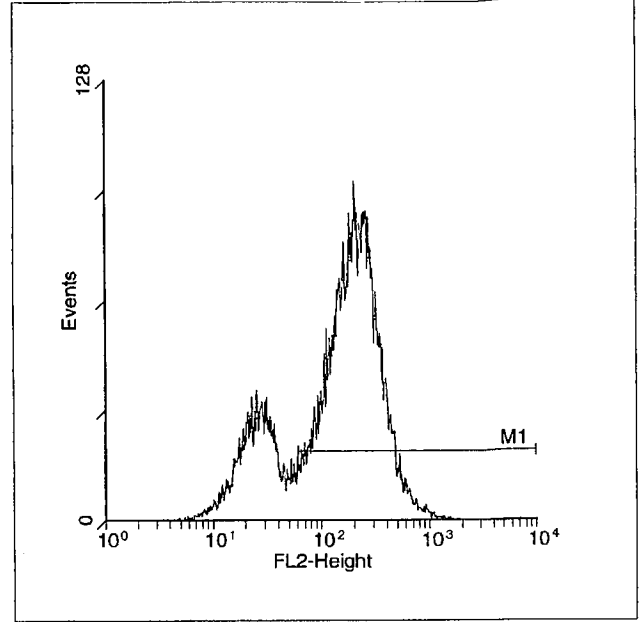
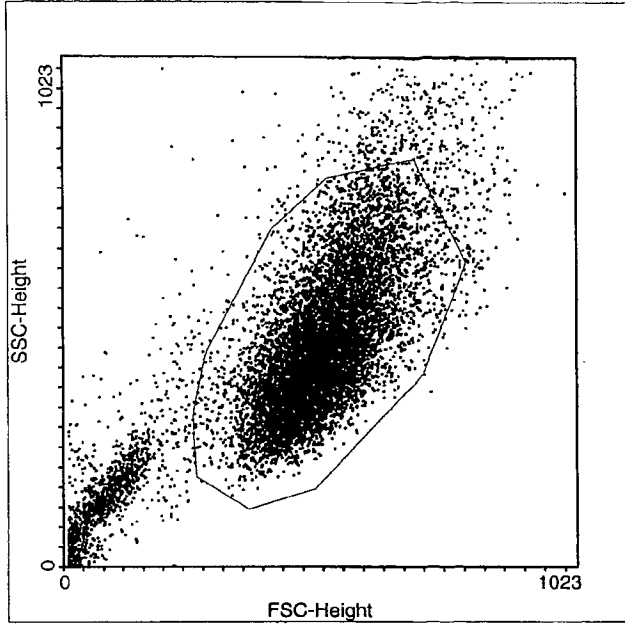
Ratio:  $\frac{67.42}{32.58} = 2.1$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 20 14:58:14 2002  
 Gates: R1

Project: Experiment:  
 File: 052002 Sample: 10Gy/0Gy(DiI)  
 Date: 20-May-2 Parameters: 1  
 Total Events 20000 Gated Events 16383 81.92%

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	16383	81.92	100.00	122.23	21.73	101,264.165
	1	457,1023	12291	61.46	<u>75.02</u>	205.71	10.24	101,264.165



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 20 14:58:51 2002

Gates: R1

Project: Experiment:

File: 052002 Sample: 10Gy/0Gy (DiI)

Date: 20-May-2 Parameters: 1

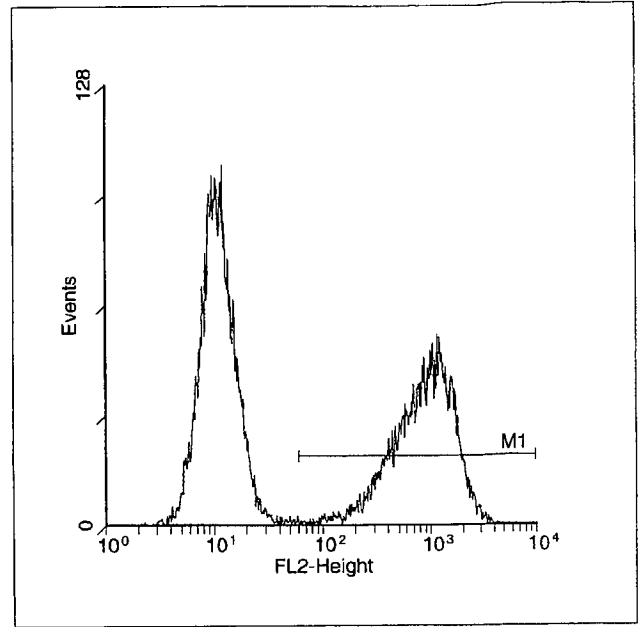
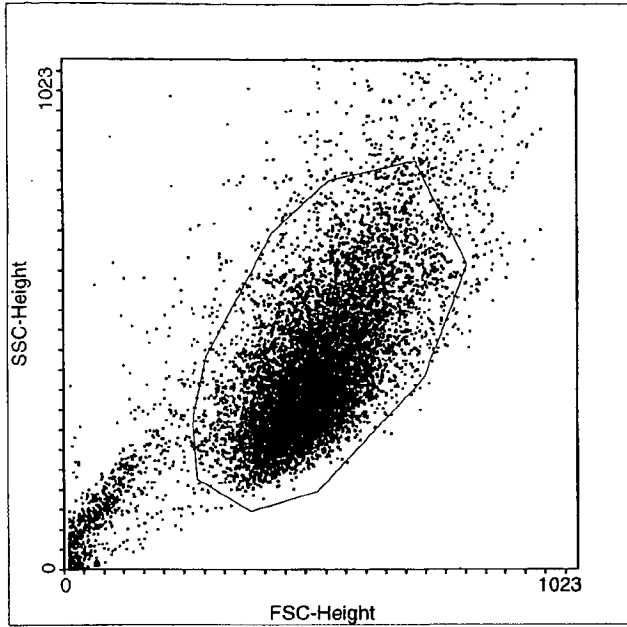
Total Events 20000 Gated Events 16329 81.64%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0,1023	16329	81.65	100.00	124.28	21.26	102,205.353
	1	457,1023	12465	62.33	76.34	203.24	10.12	102,205.353

+ 75.02  
 151.36 / 2  
 75.7





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 20 14:59:56 2002

Gates: R1

Project: Experiment: 0/5Gy+0/10Gy(DiI)

File: 052002 Sample: 0/5Gy+0/10Gy(DiI)

Date: 20-May-2 Parameters: 1

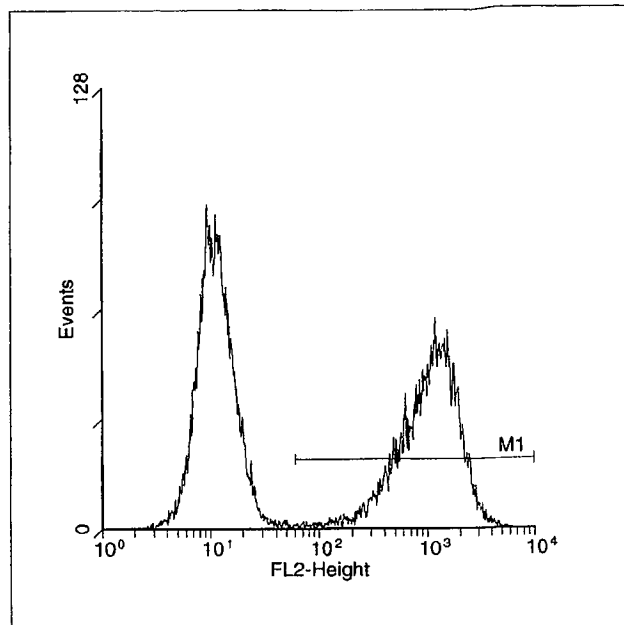
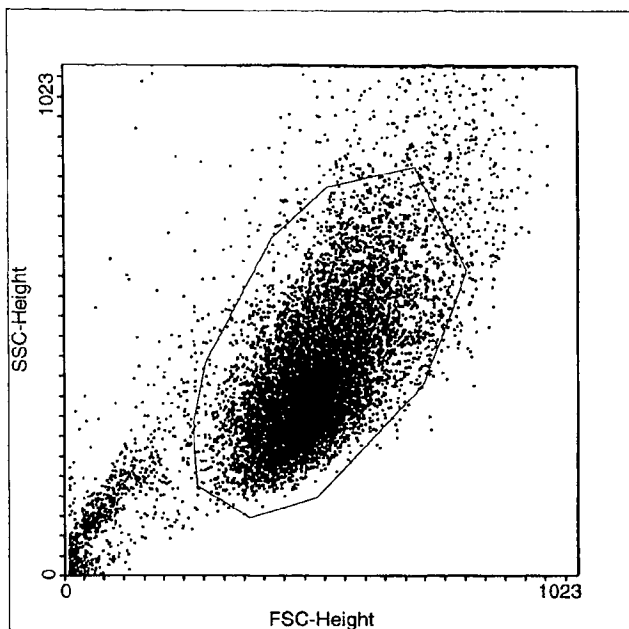
Total Events 20000 Gated Events 17429 87.14%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	17429	87.15	100.00	79.31	50.65	122,12.2983
	1	457,1023	7972	39.86	45.74	823.14	10.04	64,1144.44

0Gy/10Gy (DiI)

$$\text{Ratio} = \frac{54.3}{45.7} \approx 1.19$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon May 20 15:00:53 2002

Gates: R1

Project: Experiment:  
 File: 052002 Sample: 0/5Gy+0/10Gy (DiI)

Date: 20-May-2 Parameters: 1

Total Events 20000 Gated Events 17106 85.53%

System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	17106	85.53	100.00	97.47	50.16	101,9.47464
	1	457,1023	8261	41.31	48.29	979.60	9.75	65,1186.37

+45.74

94.03 | 2  
 (47.0)

Ratio:  $\frac{53}{47} = 1.13$

Coulter Counts  
~~Hemacytom.~~

Abs. # Ratio:  $\frac{9.23 \times 10^5}{8.97 \times 10^5} = 1.03$

Hemacytometer  
~~Coulter Counts~~

Abs # Ratio:  $\frac{677 \times 10^3}{474 \times 10^3} = 1.43$

CALCULATION  
FOR  
PROLIF. ACTIVITY

**Bogdan**

---

**From:** "Bogdan I. Gerashchenko"  
**To:** "Roger W. Howell" <rwhowell@attglobal.net>  
**Sent:** Wednesday, May 22, 2002 12:01 PM  
**Subject:** Re: today  
 Greetings to you too, Dr. Roger,

Yesterday we discussed on how to convert percentages to absolute numbers. Here are simple calculations.

Ratio of percentages of the total cell populations in the co-cultures (i.e.,  $\frac{0\text{Gy}(X1)+5\text{Gy}(Y1)}{0\text{Gy}(X2)+10\text{Gy}(Y2)}$ ) is  $\frac{53\%}{47\%} = 1.13$ .

where  $X1(67.4\%)+Y1(32.6\%) = 100$ , therefore  $\frac{100}{0\text{Gy}(X2)+10\text{Gy}(Y2)} = 1.13$  or  $0\text{Gy}(X2)+10\text{Gy}(Y2) = 88.5$

Thus, converted  $X2 = \frac{88.5 \times 75.7\%}{100\%}(0\text{Gy}(X2)) = 67$  events and  
 converted  $Y2 = \frac{88.5 \times 24.3\%}{100\%}(0\text{Gy}(Y2)) = 21.5$  events.

These preliminary data shows that irradiated cells do not affect proliferation of non-irradiated cells in a dose-dependent manner (i.e. 67.4 events in 0Gy/5Gy co-culture and 67 events in 0Gy/10Gy co-culture).

Now I am seriously thinking about paperwork after performing few experiments. I propose the title of the article as follows: "Flow Cytometry As a Strategy to Study The Co-Culture Systems in Radiation Research".

I wish you to overcome a stomach virus disease by tomorrow. Take care of yourself.

Kind regards,  
 Bogdan

----- Original Message -----

**From:** Roger W. Howell  
**To:** netipv@umdnj.edu ; Bogdan I. Gerashchenko ; detolesm@umdnj.edu  
**Sent:** Wednesday, May 22, 2002 8:32 AM  
**Subject:** today

Greetings all,

I have a stomach virus that will keep me home today. If you have any questions, please feel free to call or email. I'll see you in the morning.

Roger

5/22/2002

B002590

5/23/02

Analysis of Bogdan's 5/20/02 Data

Let  $x_1$  = number of 0Gy cells in 0/5

$y_1$  = " 5Gy "

$x_2$  = " 0Gy 0/10

$y_2$  = " 10Gy "

Data 1  $\frac{x_1}{x_1 + y_1} = 0.674$

Data 2  $\frac{x_2}{x_2 + y_2} = 0.757$

Data 3  $\frac{x_2 + y_2}{(x_1 + y_1) + (x_2 + y_2)} = 0.47$

$\therefore \frac{x_1 + y_1}{x_2 + y_2} = \frac{0.53}{0.47} = 1.13$  As per Bogdan

$\frac{x_1}{x_2} = \frac{0.674 (x_1 + y_1)}{0.757 (x_2 + y_2)} = \frac{0.674}{0.757} (1.13) = 1.006$

$$\frac{Y2}{Y1}$$

$$\text{Data 1} \quad \frac{Y1}{X1+Y1} = 0.326$$

$$\text{Data 2} \quad \frac{Y2}{X2+Y2} = \cancel{0.175} 0.243$$

$$\text{Data 3} \quad \frac{X2+Y2}{(X1+Y1) + (X2+Y2)} = 0.47$$

$$\frac{X1+Y1}{X2+Y2} = 1.13$$

$$\frac{Y2}{Y1} = \frac{\cancel{X1+Y1} (X2+Y2) \cancel{.175} .243}{\cancel{X2+Y2} (X1+Y1) .326}$$

$$\frac{Y2}{Y1} = \frac{.243}{.326} \frac{1}{1.13} = 0.66$$

From: ISAC Headquarters <isachq2@isac-net.org>

Subject: Abstract deadline: 19 October!

Date: Thu, 11 Oct 2001 12:14:46 -0500

**Time is running out!**

Only two weeks remain to submit your abstract for oral or poster presentation at the **ISAC XXI International Congress** in San Diego, 4-9 May 2002. **The deadline is 19 October 2001.** We encourage investigators in all aspects of cytometry to submit abstracts; XXI Congress themes include biological sciences, clinical sciences, and established and emerging cytometric technologies.

**How to submit an abstract:**

The ISAC XXI Abstract Submission Web site can be accessed directly at: <http://ams.cos.com/cgi-bin/conferenceToolkit/displayToolkit?institutionId=694&meetingId=64> or go to the ISAC Web site at <http://www.isac-net.org>, click on the green Congress button and follow the Call for Abstracts.

ISAC members have received their log-in and password codes. All others should use "Create a New Account" on the submission log-in page.

**Tables and graphical images (GIF and JPEG files types)** may be included as part of abstract submissions. Detailed instructions for abstract submission are provided on the Web site. All graphics will appear in black and white in the printed Congress supplement.

**Students:**

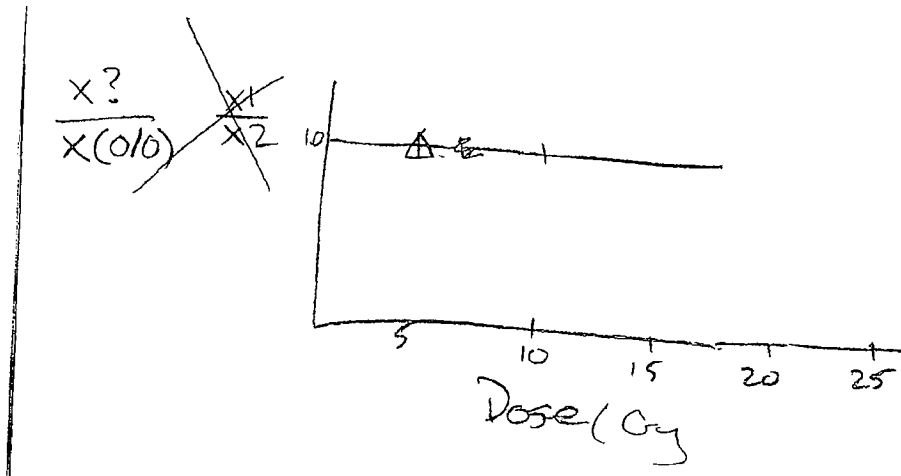
ISAC has established a fund for students whose abstracts have been accepted for either oral or poster presentation at the International Congress and who require financial aid to attend the Congress. Interested students should submit an application online as part of the abstract submission process.

**Awards:**

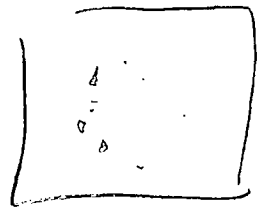
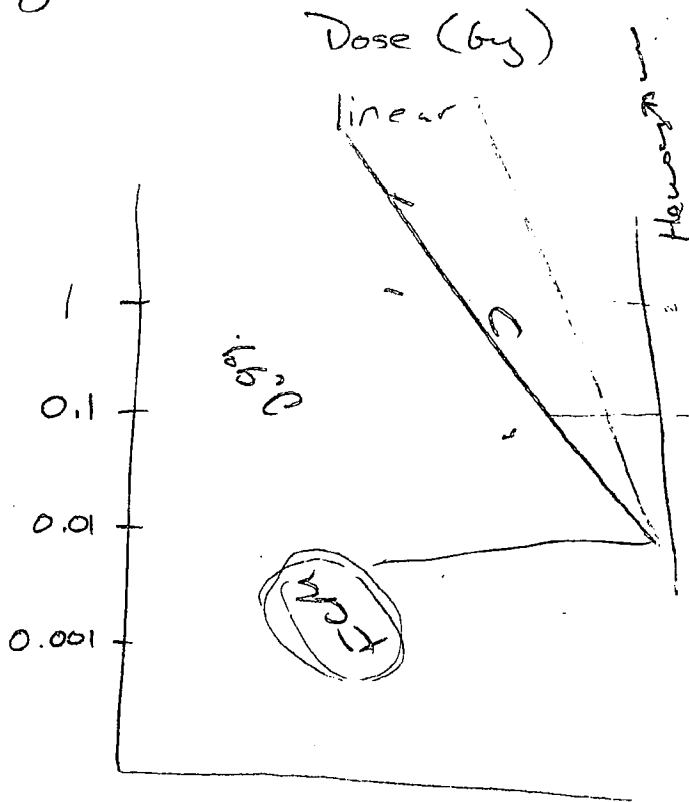
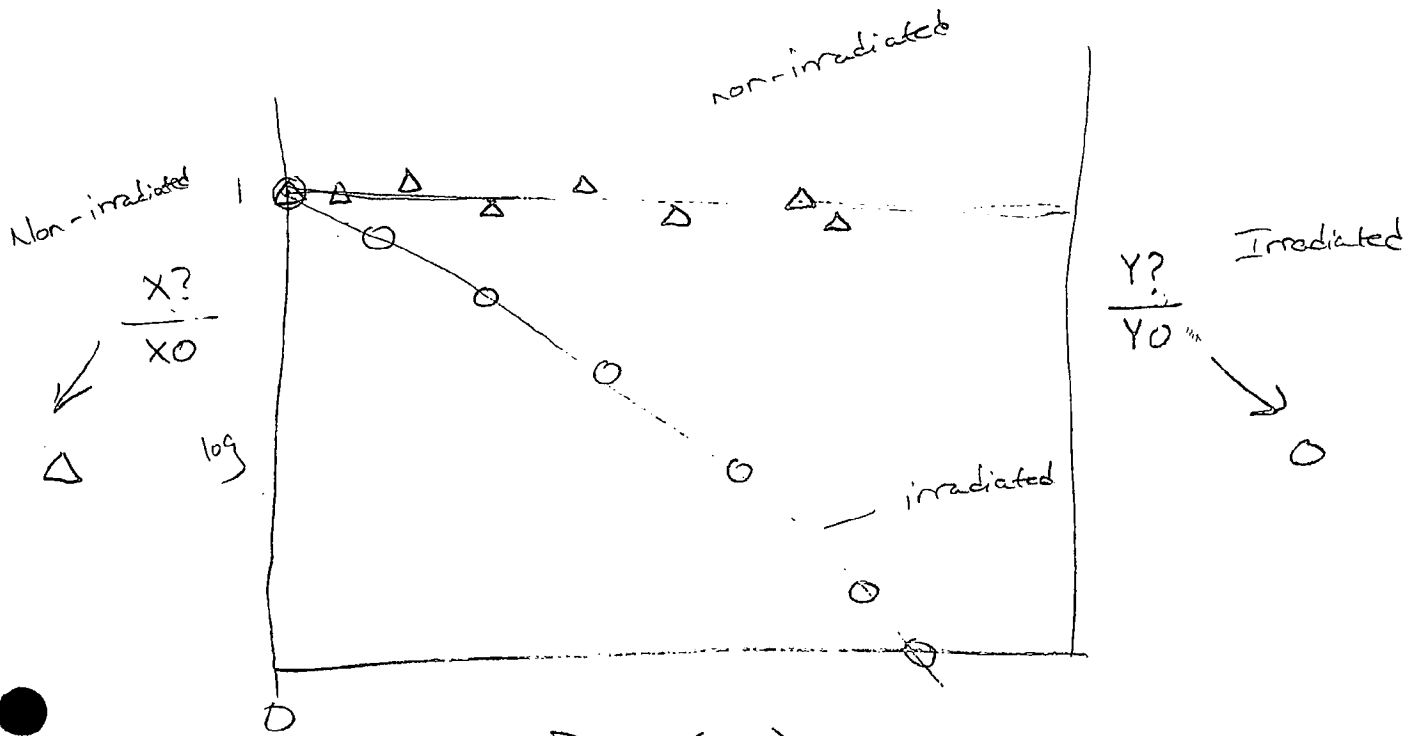
Eligible ISAC members are encouraged to compete for the ISAC New Investigator/Student Awards. Complete information about the awards and eligibility requirements is available on the ISAC Web site and as part of the abstract submission system.

If it is not possible to submit your abstract electronically, contact Nicki Patti at ISAC headquarters [npatti@isac-net.org](mailto:npatti@isac-net.org) or 847-205-4722 ext. 214 for instructions regarding alternative submission methods. **All abstracts must be submitted by 19 October 2001.**

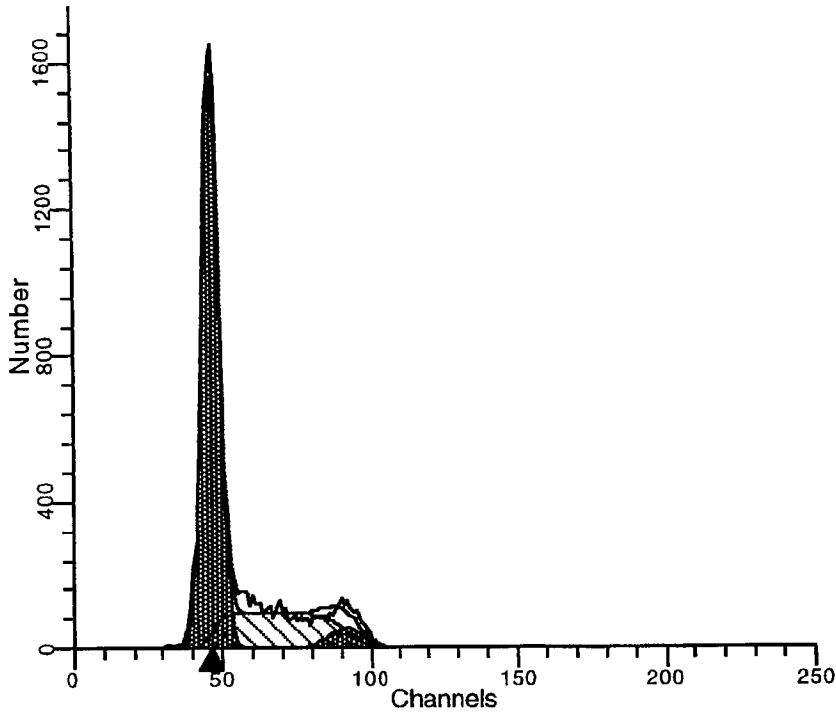
We'll see you in sunny San Diego next May!







	Label	A
Label		



Date acquired: 21-May-2  
 File: 052102 .001  
 Source: WB 0Gy  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

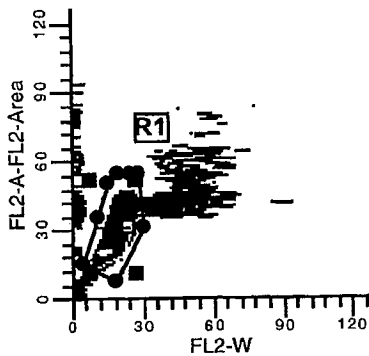
DIPLOID: 100.00 %  
 Dip G0-G1: 69.16 % at 46.28  
 Dip G2-M: 4.70 % at 91.41  
 Dip S: 26.14 % G2/G1: 1.98  
 Dip %CV: 6.14

Total S-Phase: 26.14 %

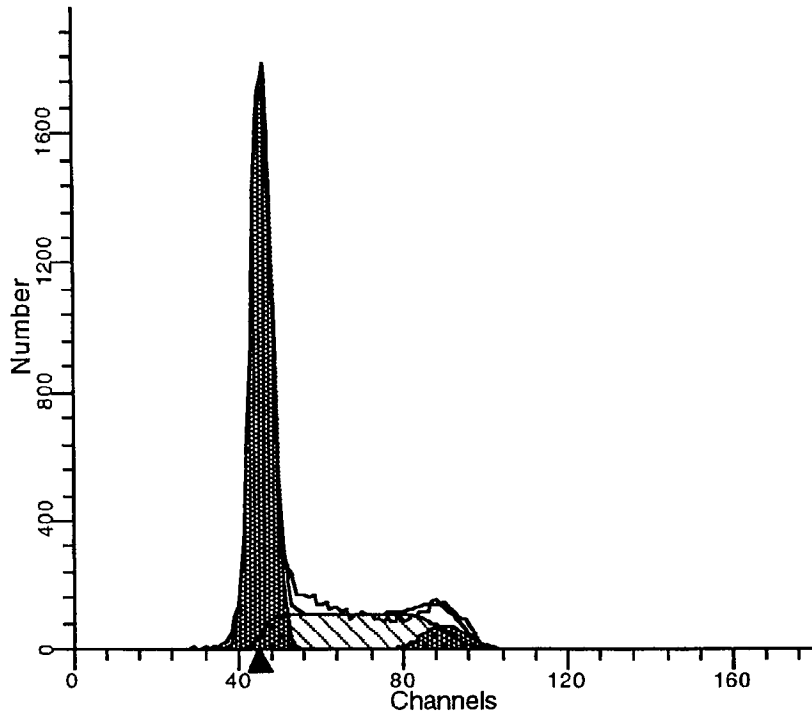
Extra Pop: %  
 Debris: 0.74 %  
 Aggregates: 0.00 %  
 Modeled Events: 16410  
 RCS: 2.023

Diploid B.A.D.: 0.36 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 21-May-2  
 File: 052102 .002  
 Source: WB 0Gy  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

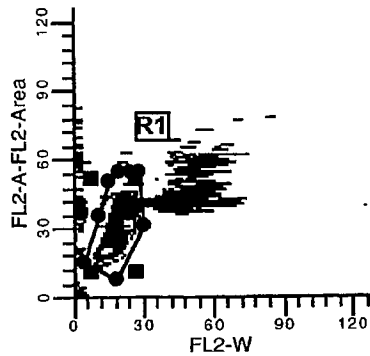
DIPLOID: 100.00 %  
 Dip G0-G1: 65.60 % at 45.71  
 Dip G2-M: 5.46 % at 90.10  
 Dip S: 28.94 % G2/G1: 1.97  
 Dip %CV: 5.54 *Average: 27.54*

Total S-Phase: 28.94 %

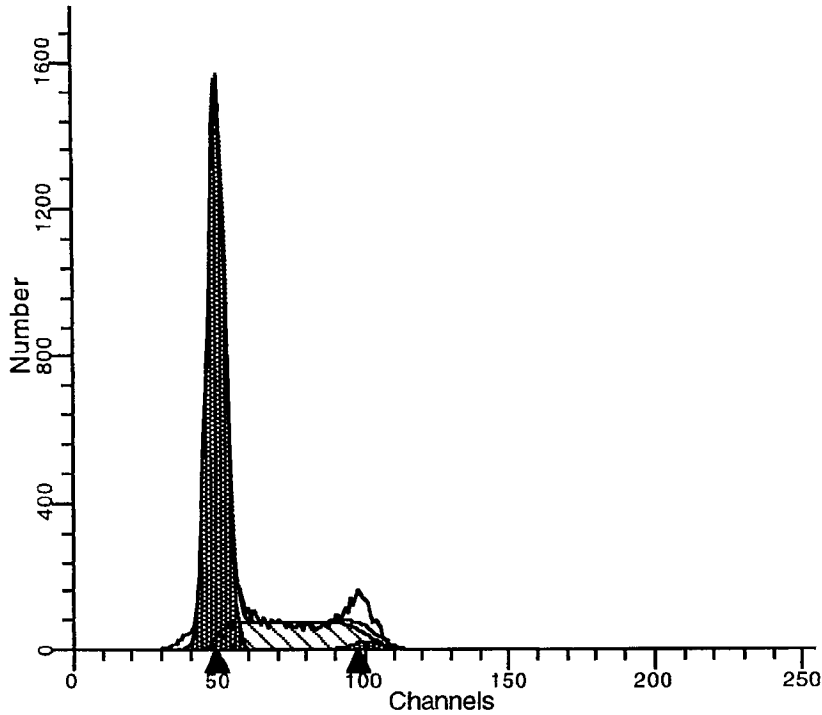
Extra Pop: %  
 Debris: 0.38 %  
 Aggregates: 0.00 %  
 Modeled Events: 17182  
 RCS: 2.428

Diploid B.A.D.: 0.15 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 21-May-2  
 File: 052102 .003  
 Source: WB-5 Gy  
 Case: PATIENT-ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

DIPLOID: 100.00 %  
 Dip G0-G1: 72.47 % at 49.23  
 Dip G2-M: 2.06 % at 101.19  
 Dip S: 25.47 % G2/G1: 2.06  
 Dip %CV: 6.11

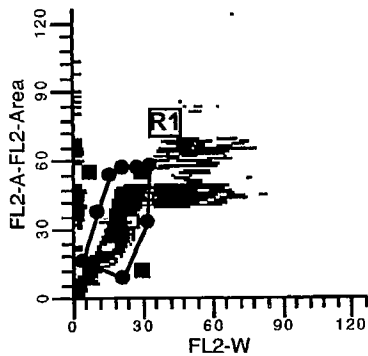
Total S-Phase: 25.47 %

2

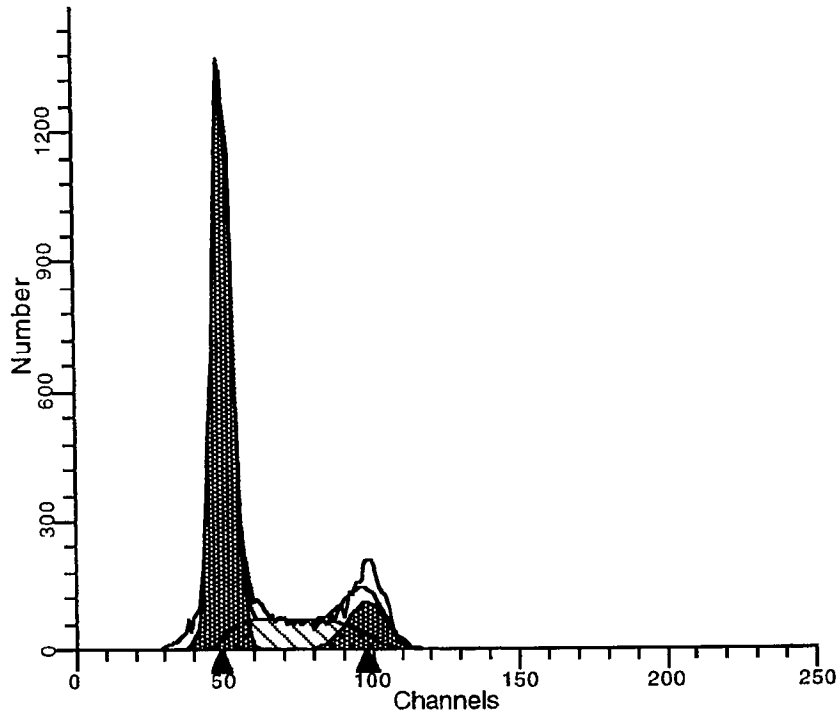
Extra Pop: %  
 Debris: 0.36 %  
 Aggregates: 0.00 %  
 Modeled Events: 15984  
 RCS: 3.470

Diploid B.A.D.: 0.05 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 21-May-2  
 File: 052102 .004  
 Source: WB 10 Gy  
 Case: PATIENTID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

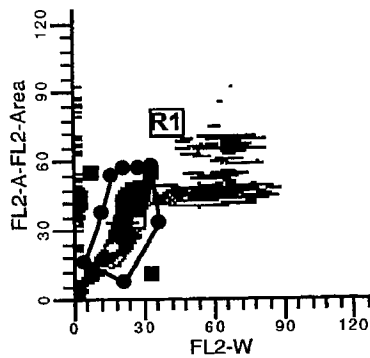
DIPLOID: 100.00 %  
 Dip G0-G1: 68.75 % at 49.67  
 Dip G2-M: 11.32 % at 97.80  
 Dip S: 19.92 % G2/G1: 1.97  
 Dip %CV: 6.76

Total S-Phase: 19.92 %

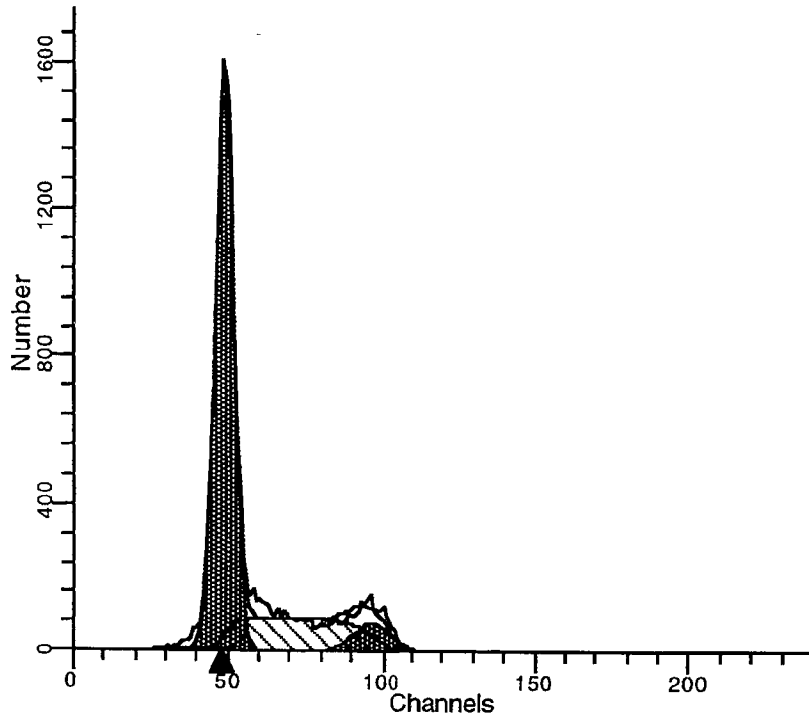
Extra Pop: %  
 Debris: 0.48 %  
 Aggregates: 0.00 %  
 Modeled Events: 16197  
 RCS: 3.480

Diploid B.A.D.: 0.17 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 21-May-2  
 File: 052102 .005  
 Source: (WB 0/5 Gy)  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

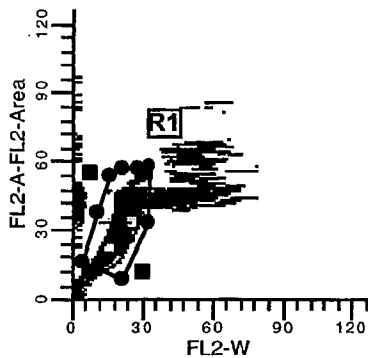
DIPLOID: 100.00 %  
 Dip G0-G1: 68.58 % at 48.76  
 Dip G2-M: 6.73 % at 96.30  
 Dip S: 24.69 % G2/G1: 1.98  
 Dip %CV: 6.11

Total S-Phase: 24.69 %

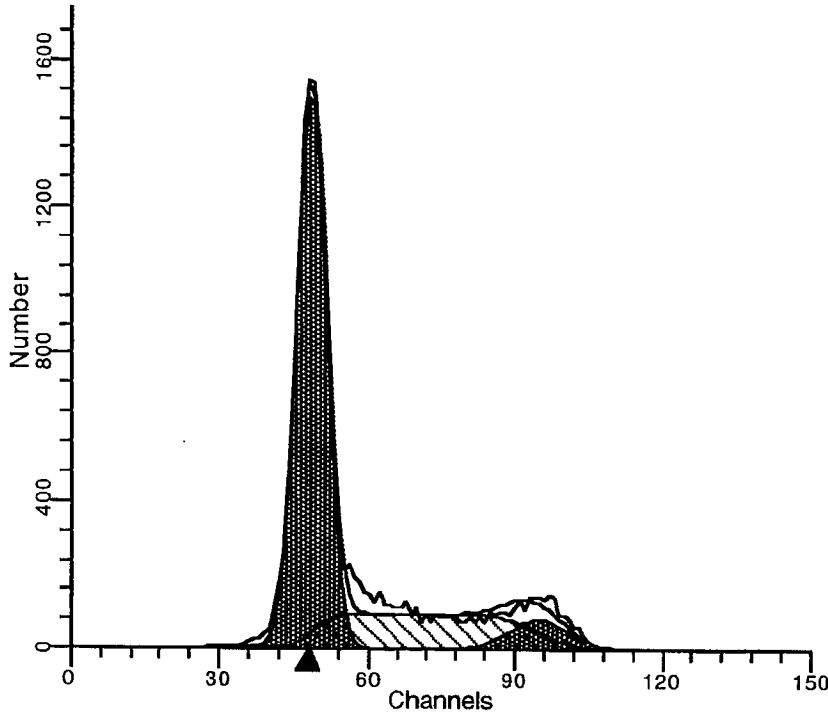
Extra Pop: %  
 Debris: 1.29 %  
 Aggregates: 0.00 %  
 Modeled Events: 16800  
 RCS: 1.989

Diploid B.A.D.: 0.62 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFitLT V2.0(PMac)



Date acquired: 21-May-2  
 File: 052102 .006  
 Source: WB 0/10 Gy  
 Case: PATIENT ID  
 Analysis type: Manual analysis  
 Prep: Fresh/Frozen

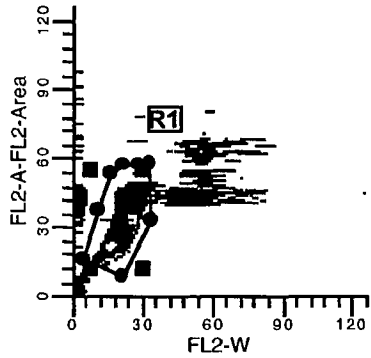
DIPLOID: 100.00 %  
 Dip G0-G1: 66.67 % at 48.39  
 Dip G2-M: 7.03 % at 94.77  
 Dip S: 26.31 % G2/G1: 1.96  
 Dip %CV: 6.08

Total S-Phase: 26.31 %

Extra Pop: %  
 Debris: 0.91 %  
 Aggregates: 0.00 %  
 Modeled Events: 17055  
 RCS: 3.700

Diploid B.A.D.: 0.47 % no aggs

S-Phase Assessment:  
 Tissue Type: All  
 Model type: Diploid  
 Diploid S: High  
 Calculated p-value:  $p < 0.01$   
 S-phase Boundaries: 5.0 and 10.0



ModFiILT V2.0(PMac)





## Trypan Blue

Product Nos. T 8154, T 6146 and Z 35,962-9 (H7901)

### USE OF TRYPAN BLUE STAIN AND THE HEMOCYTOMETER TO DETERMINE TOTAL CELL COUNTS AND VIABLE CELL NUMBER

Trypan Blue is one of several stains recommended for use in dye exclusion procedures for viable cell counting. This method is based on the principle that live (viable) cells do not take up certain dyes, whereas dead (non-viable) cells do. Staining facilitates the visualization of cell morphology.

**NOTE:** Trypan Blue has a greater affinity for serum proteins than for cellular protein. If the background is too dark, cells should be pelleted and resuspended in protein-free medium or salt solution prior to counting.

- 1) Prepare a cell suspension in a balanced salt solution (e.g., Hanks' Balanced Salts [HBSS], Product No. H 2513).
- 2) Transfer 0.5 ml of 0.4% Trypan Blue solution (w/v) to a test tube. Add 0.3 ml of HBSS and 0.2 ml of the cell suspension (dilution factor = 5) and mix thoroughly. Allow to stand for 5 to 15 minutes.

**NOTE:** If cells are exposed to Trypan Blue for extended periods of time, viable cells, as well as non-viable cells, may begin to take up dye.

- 3) With the cover-slip in place, use a Pasteur pipette or other suitable device to transfer a small amount of Trypan Blue-cell suspension mixture to both chambers of the hemocytometer. Carefully touch the edge of the cover-slip with the pipette tip and allow each chamber to fill by capillary action. Do not overfill or underfill the chambers.

- 4) Starting with chamber 1 of the hemocytometer, count all the cells in the 1 mm center square and four 1 mm corner squares (see **Diagram I**). Non-viable cells will stain blue. Keep a separate count of viable and non-viable cells.

**NOTE:** Count cells on top and left touching middle line of the perimeter of each square. Do not count cells touching the middle line at bottom and right sides (see **Diagram II**).

## Product Information

- 5) Repeat this procedure for chamber 2.

**NOTE:** If greater than 10% of the cells appear clustered, repeat entire procedure making sure the cells are dispersed by vigorous pipetting in the original cell suspension as well as the Trypan Blue-cell suspension mixture. If less than 200 or greater than 500 cells (i.e., 20-50 cells/square) are observed in the 10 squares, repeat the procedure adjusting to an appropriate dilution factor.

- 6) Withdraw a second sample and repeat count procedure to ensure accuracy.

- 7) **CELL COUNTS** — Each square of the hemocytometer, with cover-slip in place, represents a total volume of 0.1 mm<sup>3</sup> or 10<sup>-4</sup> cm<sup>3</sup>. Since 1 cm<sup>3</sup> is equivalent to approximately 1 ml, the subsequent cell concentration per ml (and the total number of cells) will be determined using the following calculations:

**CELLS PER ml** = the average count per square × dilution factor × 10<sup>4</sup> (count 10 squares)

**Ex:** If the average count per square is 45 cells × 5 × 10<sup>4</sup> = 2.25 × 10<sup>6</sup> cells/ml.

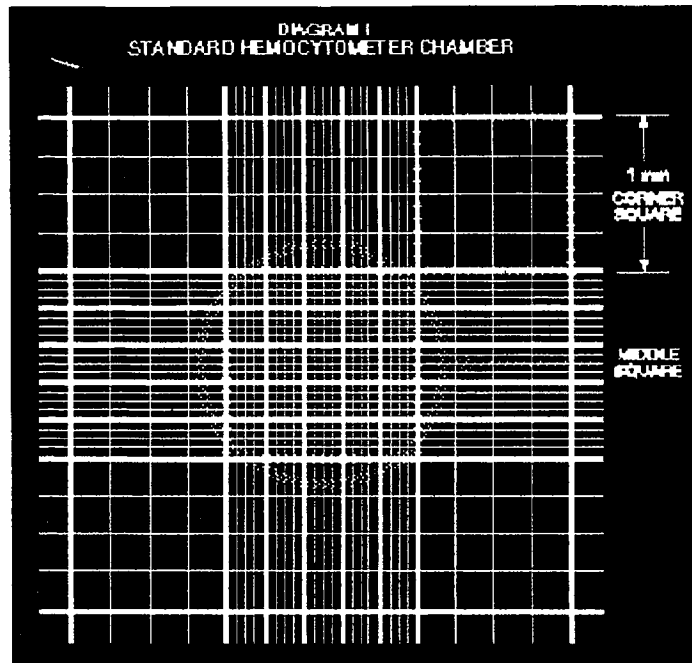
**TOTAL CELLS** = cells per ml × the original volume of fluid from which cell sample was removed.

**Ex:** 2.25 × 10<sup>6</sup> (cells/ml) × 10 ml (original volume) = 2.25 × 10<sup>7</sup> total cells.

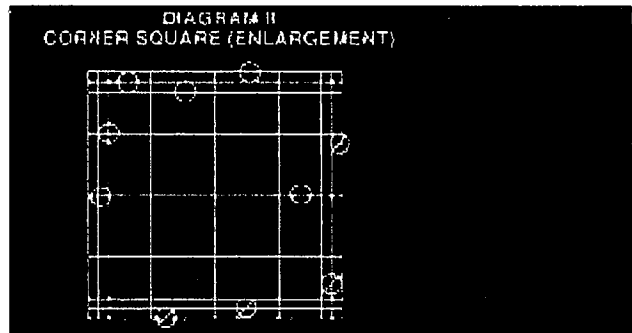
- 8) **CELL VIABILITY (%)** = total viable cells (unstained) ÷ total cells (stained and unstained) × 100.

**Ex:** If the average count per square of unstained (viable) cells is 37.5, the total viable cells = [37.5 × 5 × 10<sup>4</sup>] viable cells/ml × 10 ml (original volume) = 1.875 × 10<sup>7</sup> viable cells. Cell viability (%) = 1.875 × 10<sup>7</sup> (viable cells) ÷ 2.25 × 10<sup>7</sup> (total cells) × 100 = 83% viability.

**Trypan Blue continued**



The circle indicates the approximate area covered at 100x microscope magnification (10x ocular and 10x objective). Include cells on top and left touching middle line (O). Do not count cells touching middle line at bottom and right (e). Count 4 corner squares and middle square in both chambers (one chamber represented here).



Count cells on top and left touching middle line (O). Do not count cells touching middle line at bottom and right (e).



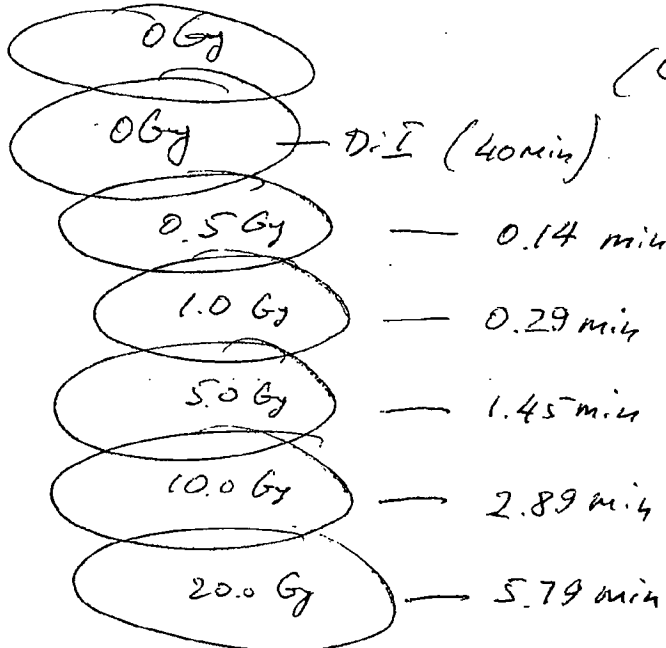
70-CULTURE

MAY 28, 2002

BYSTANDER EFFECTS (β-IRRADIATION)

W13 (19) 100% confluency

IRRADIATION TIME: 8:20  
(ONE SOURCE: 345.59 Rad/min)

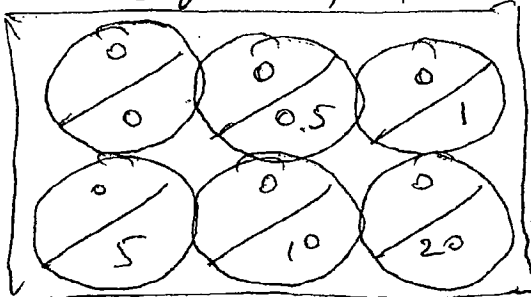
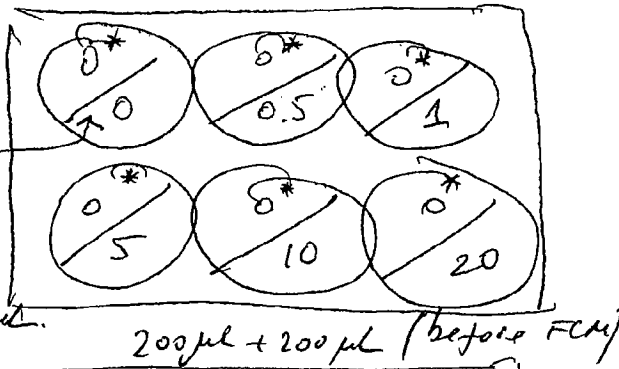


PLATING TIME: 10:50

200 μL / 200 μL

\* 0 Gy - D: I stained.

Added less than 200 μL



For subsequent staining with D: I,

- 1) 0/0 (200 μL) + 0/0.5 (200 μL)
- 2) 0/0 (200 μL) + 0/1 (200 μL)
- 3) 0/0 (200 μL) + 0/5 (200 μL)
- 4) 0/0 (200 μL) + 0/10 (200 μL)
- 5) 0/0 (200 μL) + 0/20 (200 μL)

Note: PLATE 100 μL next time.

No. plated cells

$$\begin{array}{l} 232 \\ 238 \end{array} \left. \vphantom{\begin{array}{l} 232 \\ 238 \end{array}} \right\} 470 \times 10^3 \text{ cells/mL} \\ + 0.5 \text{ mL} \rightarrow 705 \times 10^3 \text{ cells}$$

$$\begin{array}{l} 1.1 \text{ mL contains } 705 \times 10^3 \text{ cells} \\ 0.4 \text{ mL} \longrightarrow 256 \times 10^3 \text{ cells} \\ 0.2 \text{ mL} \longrightarrow 128 \times 10^3 \text{ cells} \end{array}$$

↓  
Check again!  
in next experiments.

MAY 29, 2002

HEMOCYTOMETER COUNTS.

$$0 \text{ Gy} / 0 \text{ Gy: } \begin{array}{l} 263 \\ 314 \end{array} \left. \vphantom{\begin{array}{l} 263 \\ 314 \end{array}} \right\} 577 \times 10^3 \text{ cells/mL} \\ + 0.5 \text{ mL} \rightarrow 865 \times 10^3 \text{ cells}$$

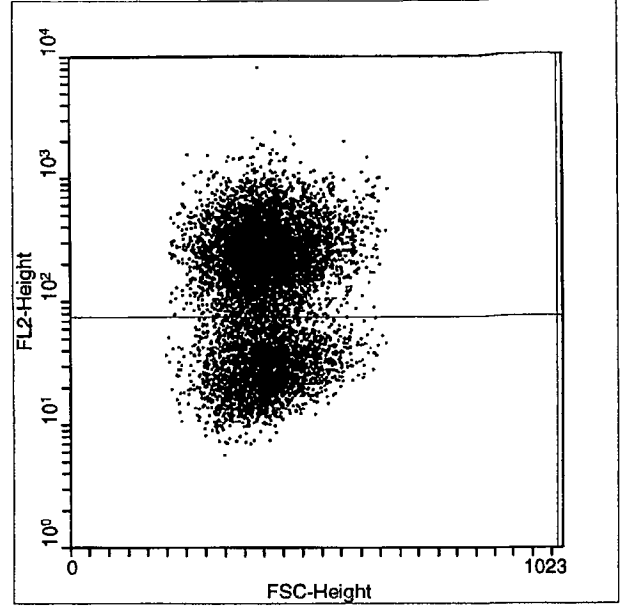
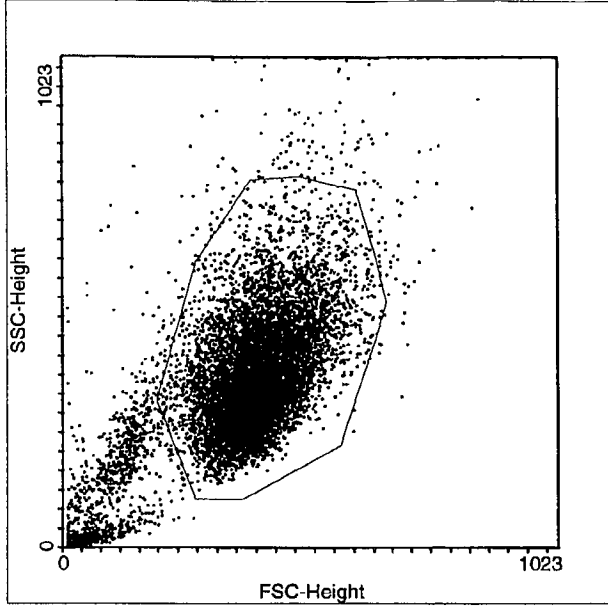
$$0 \text{ Gy} / 0.5 \text{ Gy: } \begin{array}{l} 284 \\ 355 \end{array} \left. \vphantom{\begin{array}{l} 284 \\ 355 \end{array}} \right\} 639 \times 10^3 \text{ cells/mL} \\ + 0.5 \text{ mL} \rightarrow 959 \times 10^3 \text{ cells}$$

$$0 \text{ Gy} / 1 \text{ Gy: } \begin{array}{l} 333 \\ 297 \end{array} \left. \vphantom{\begin{array}{l} 333 \\ 297 \end{array}} \right\} 630 \times 10^3 \text{ cells/mL} \\ + 0.5 \text{ mL} \rightarrow 945 \times 10^3 \text{ cells}$$

$$0 \text{ Gy} / 5 \text{ Gy: } \begin{array}{l} 244 \\ 247 \end{array} \left. \vphantom{\begin{array}{l} 244 \\ 247 \end{array}} \right\} 491 \times 10^3 \text{ cells/mL} \\ + 0.5 \text{ mL} \rightarrow 737 \times 10^3 \text{ cells}$$

$$0 \text{ Gy} / 10 \text{ Gy: } \begin{array}{l} 262 \\ 244 \end{array} \left. \vphantom{\begin{array}{l} 262 \\ 244 \end{array}} \right\} 506 \times 10^3 \text{ cells/mL} \\ + 0.5 \text{ mL} \rightarrow 759 \times 10^3 \text{ cells}$$

$$0 \text{ Gy} / 20 \text{ Gy: } \begin{array}{l} 208 \\ 239 \end{array} \left. \vphantom{\begin{array}{l} 208 \\ 239 \end{array}} \right\} 447 \times 10^3 \text{ cells/mL} \\ + 0.5 \text{ mL} \rightarrow 671 \times 10^3 \text{ cells}$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 29 14:46:54 2002

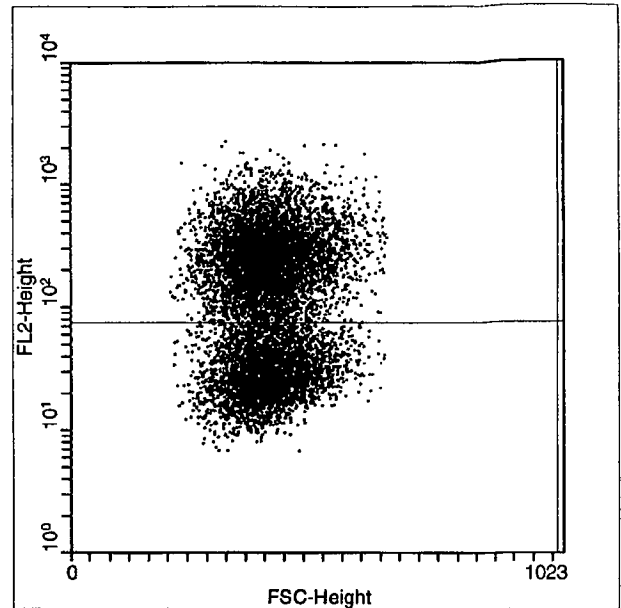
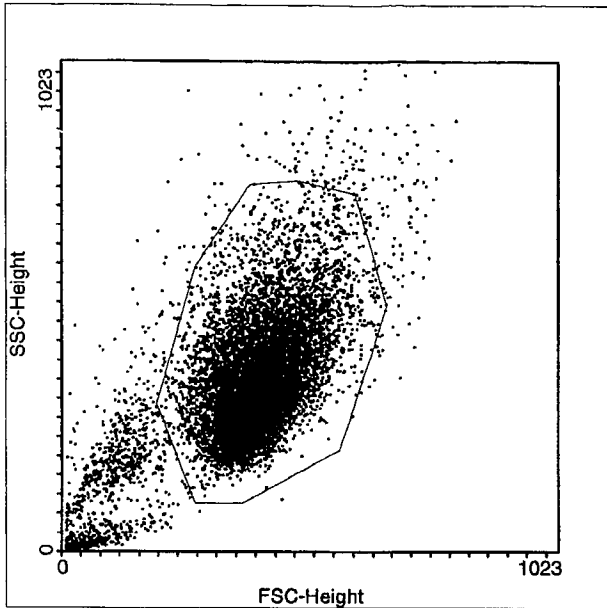
Gates: R1  
 Project: Experiment:  
 File: 052902 Sample: 0Gy(DiI)/0Gy 22h  
 Date: 29-May-2 Parameters: 7  
 Total Events 20000 Gated Events 17483 87.42%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FSC-Height(Lin) vs FL2-Height(Log)  
 Quadrant x,y: 1012,480

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	405.1	254.7	11516	57.58	65.87
2 UR	0.0	0.0	0	0.00	0.00
3 LL	402.3	29.3	5967	29.84	34.13
4 LR	0.0	0.0	0	0.00	0.00

Plated less than 200µL

Ratio should be 1.0

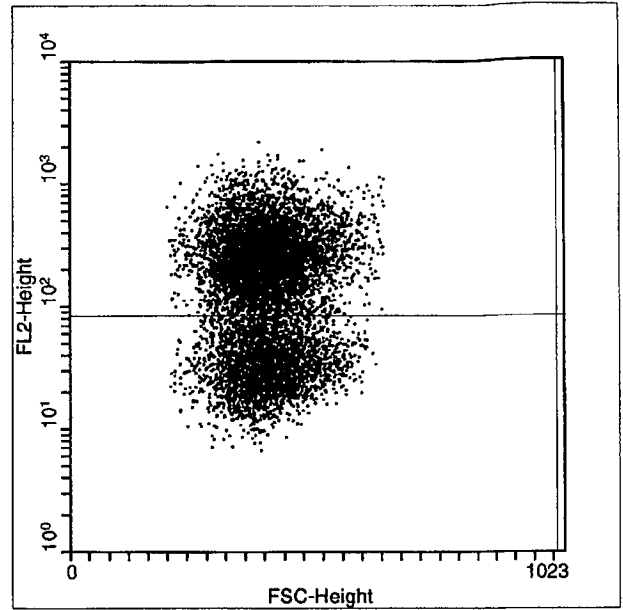
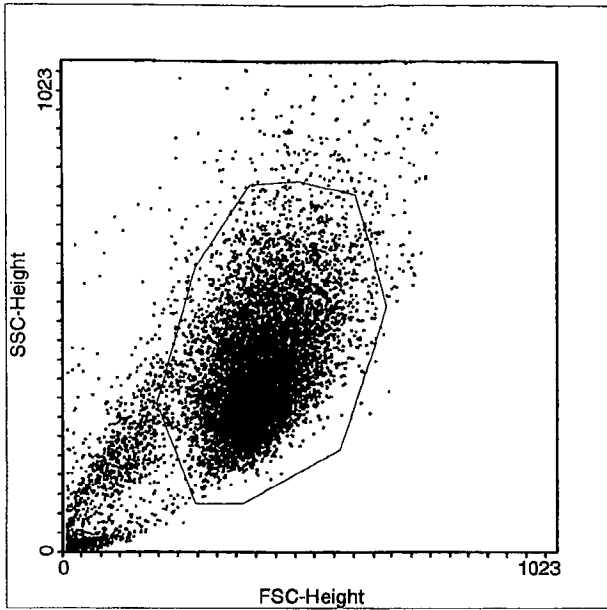


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 29 14:50:01 2002  
 Gates: R1  
 Project: Experiment:  
 File: 052902 Sample: 0Gy (DiI) / 0.5Gy 22h  
 Date: 29-May-2 Parameters: 7  
 Total Events 20000 Gated Events 17581 87.90%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FSC-Height(Lin) vs FL2-Height(Log)

Quadrant x,y: 1012,480

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	412.6	255.0	10667	53.34	60.67
2 UR	0.0	0.0	0	0.00	<del>0.00</del>
3 LL	410.1	29.0	6914	34.57	39.33
4 LR	0.0	0.0	0	0.00	0.00

1.54



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 29 14:52:54 2002

Gates: R1

Project: Experiment:

File: 052902 Sample: OGy(DiI)/1Gy 22h

Date: 29-May-2 Parameters: 7

Total Events 20000 Gated Events 17199 86.00%

System: Log Parameter Means: Geometric

Quad Stats

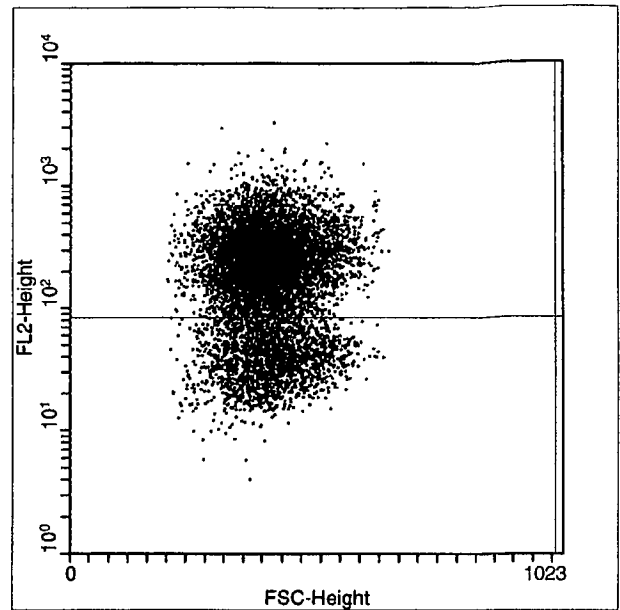
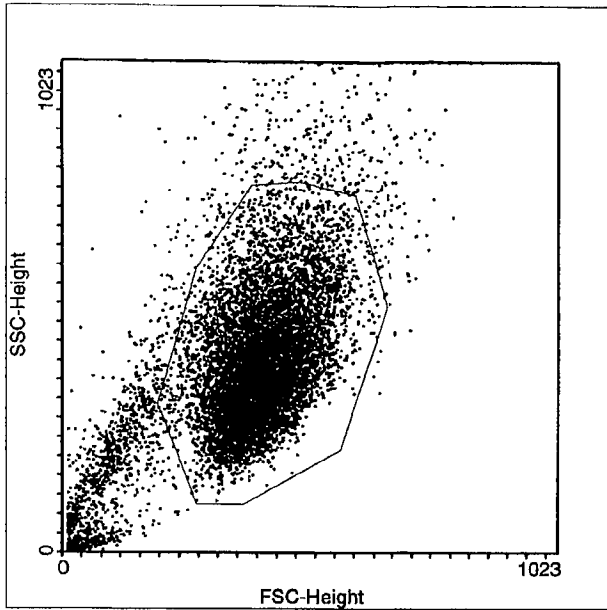
FSC-Height(Lin) vs FL2-Height(Log)

Quadrant x,y: 1008,492

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	407.8	262.8	11227	56.14	65.28
2 UR	0.0	0.0	0	0.00	<del>0.00</del>
3 LL	408.7	32.7	5972	29.86	34.72
4 LR	0.0	0.0	0	0.00	0.00

1.88





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 29 14:54:42 2002

Gates: R1

Project: Experiment:

File: 052902 Sample: OGy(DiI)/5Gy 22h

Date: 29-May-2 Parameters: 7

Total Events 20000 Gated Events 16952 84.76%

System: Log Parameter Means: Geometric

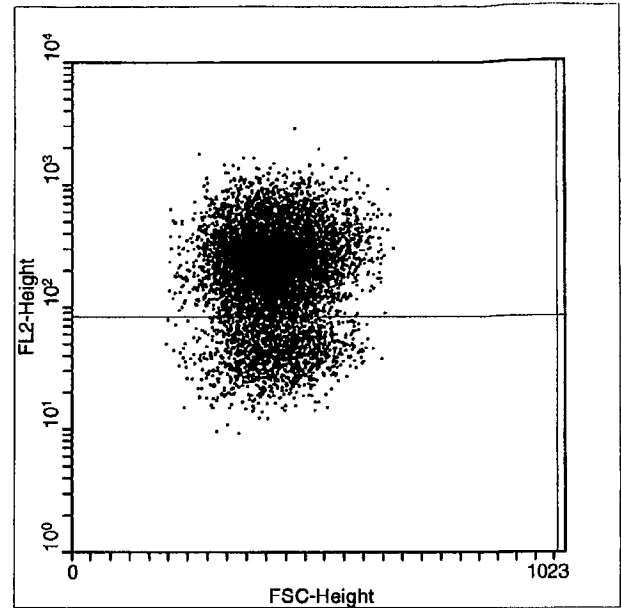
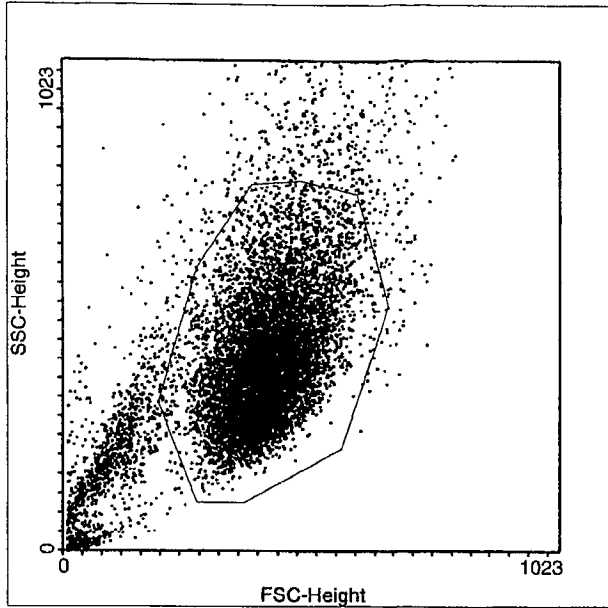
Quad Stats

FSC-Height(Lin) vs FL2-Height(Log)

Quadrant x,y: 1008,492

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	411.3	259.0	13085	65.43	77.19
2 UR	0.0	0.0	0	0.00	<del>0.00</del>
3 LL	417.3	38.3	3867	19.34	22.81
4 LR	0.0	0.0	0	0.00	0.00

3.38



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 29 14:55:48 2002

Gates: R1

Project: Experiment;

File: 052902 Sample: 0Gy(DiI)/10Gy 22h

Date: 29-May-2 Parameters: 7

Total Events 20000 Gated Events 16495 82.47%

System: Log Parameter Means: Geometric

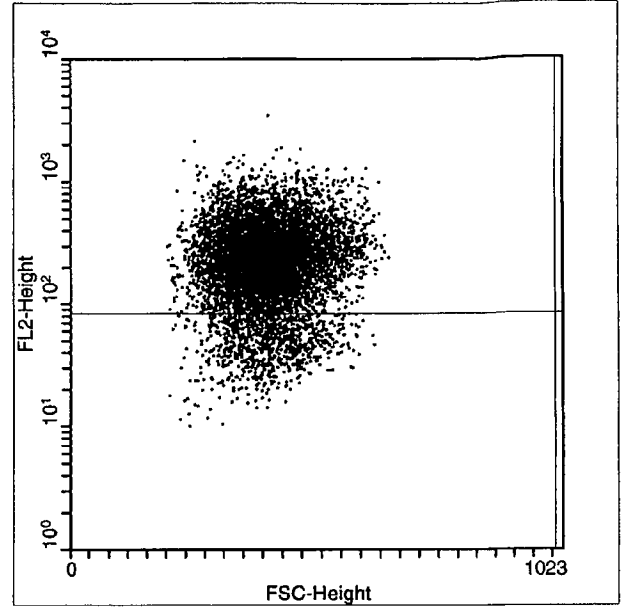
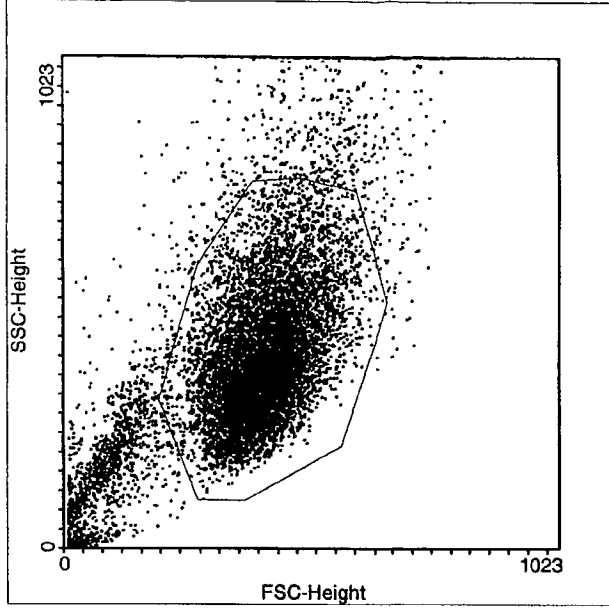
Quad Stats

FSC-Height(Lin) vs FL2-Height(Log)

Quadrant x,y: 1008,492

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	418.5	259.5	13504	67.52	81.87
2 UR	0.0	0.0	0	0.00	<del>0.00</del>
3 LL	420.2	43.5	2991	14.96	18.13
4 LR	0.0	0.0	0	0.00	0.00

4.52



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 29 14:56:29 2002

Gates: R1

Project: Experiment:

File: 052902 Sample: 0Gy(DiI)/20Gy 22h

Date: 29-May-2 Parameters: 7

Total Events 20000 Gated Events 16061 80.31%

System: Log Parameter Means: Geometric

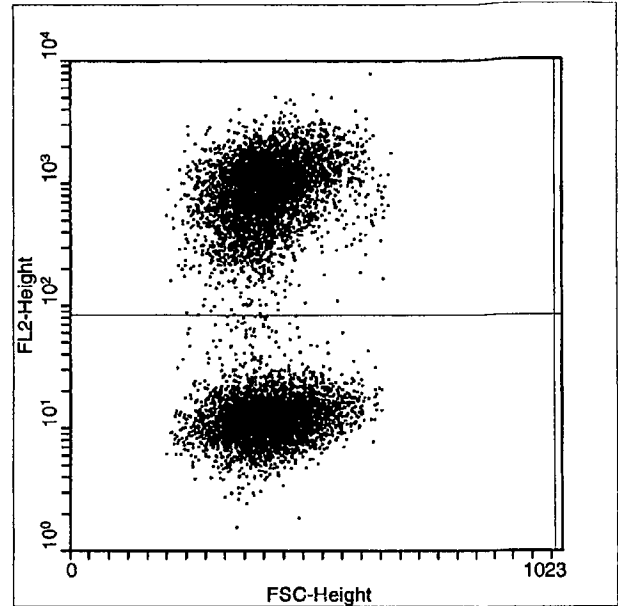
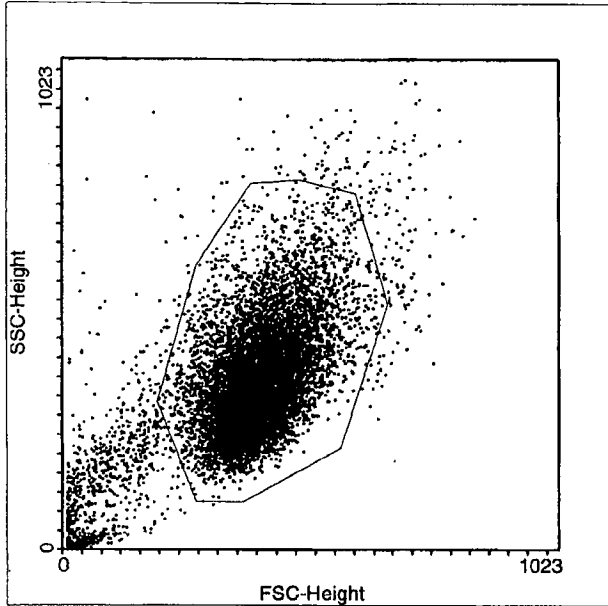
Quad Stats

FSC-Height(Lin) vs FL2-Height(Log)

Quadrant x,y: 1008,492

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	413.6	255.9	14201	71.01	88.42
2 UR	0.0	0.0	0	0.00	0.00
3 LL	412.3	47.7	1860	9.30	11.58
4 LR	0.0	0.0	0	0.00	0.00

> 7.64



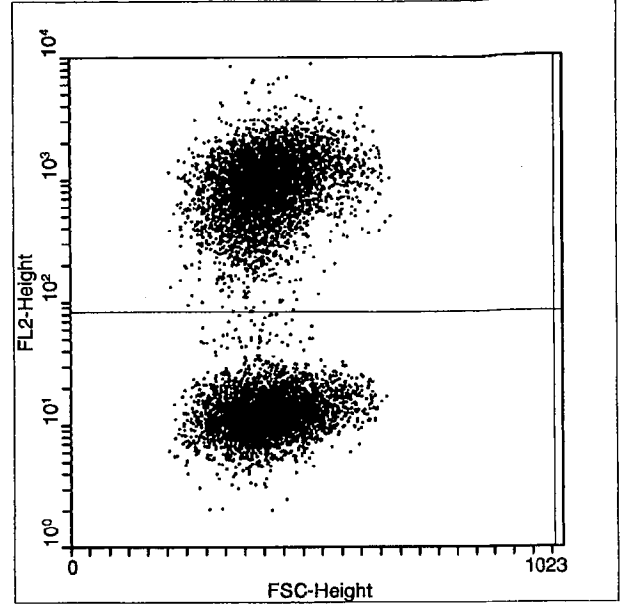
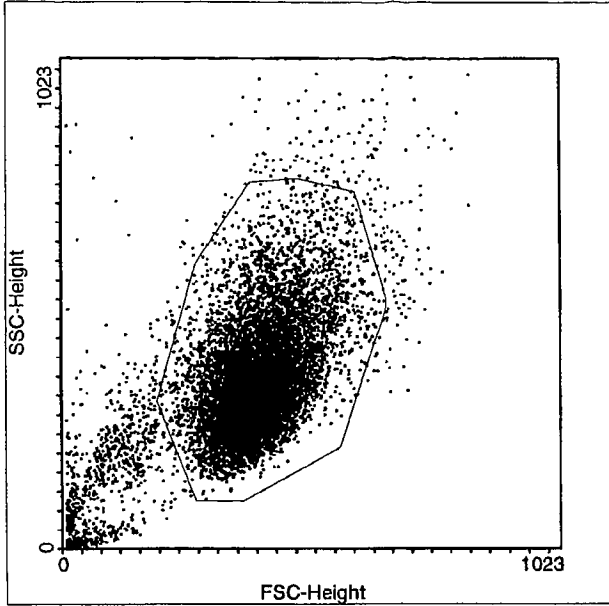
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 29 14:57:01 2002

Gates: R1  
 Project: Experiment: 5  
 File: 052902 Sample: 0Gy(DiI)+0.5Gy 22h  
 Date: 29-May-2 Parameters: 7  
 Total Events 20000 Gated Events 17898 89.49%

System: Log Parameter Means: Geometric  
 Quad Stats  
 FSC-Height(Lin) vs FL2-Height(Log)  
 Quadrant x,y: 1008,492

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	407.7	875.3	9106	45.53	50.88
2 UR	0.0	0.0	0	0.00	<del>0.00</del>
3 LL	409.4	12.0	8792	43.96	49.12
4 LR	0.0	0.0	0	0.00	0.00

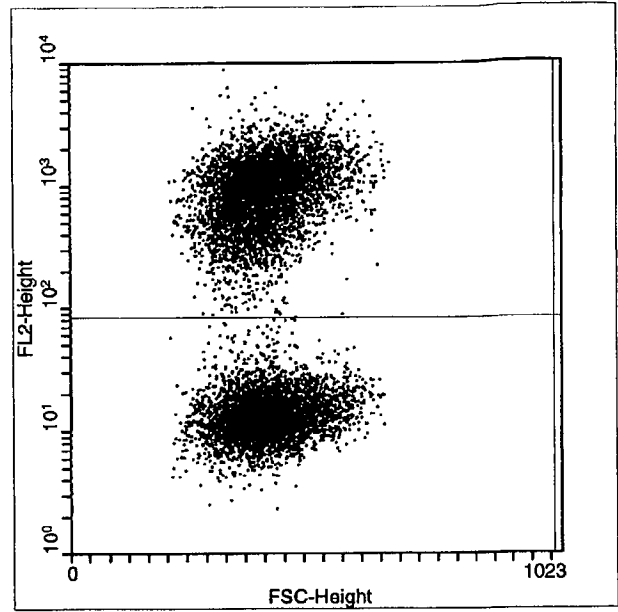
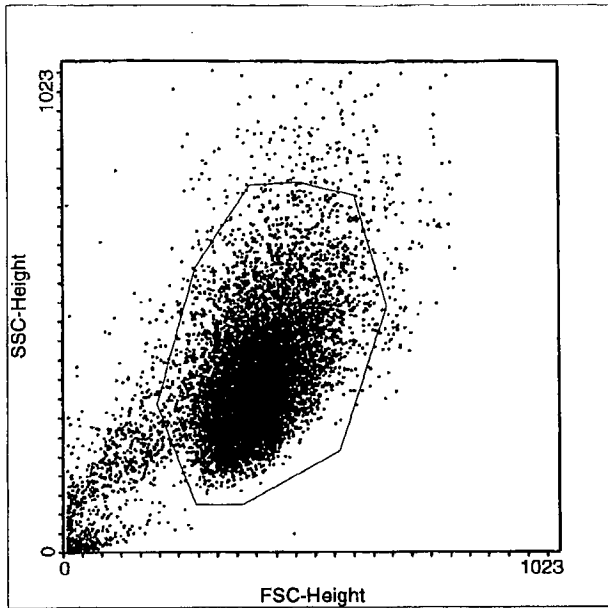
> 1.04



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 29 15:07:49 2002  
 Gates: R1  
 Project: Experiment:  
 File: 052902 Sample: 0Gy(DiI)+1Gy 22h  
 Date: 29-May-2 Parameters: 7  
 Total Events 20000 Gated Events 17694 88.47%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FSC-Height(Lin) vs FL2-Height(Log)  
 Quadrant x,y: 1008,492

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	406.8	878.9	8985	44.93	50.78
2 UR	0.0	0.0	0	0.00	<del>0.00</del>
3 LL	407.7	12.4	8709	43.55	49.22
4 LR	0.0	0.0	0	0.00	0.00

1.03



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 29 14:59:02 2002

Gates: R1

Project: Experiment:

File: 052902 Sample: 0Gy (DiI)+5Gy 22h

Date: 29-May-2 Parameters: 7

Total Events 20000 Gated Events 17698 88.49%

System: Log Parameter Means: Geometric

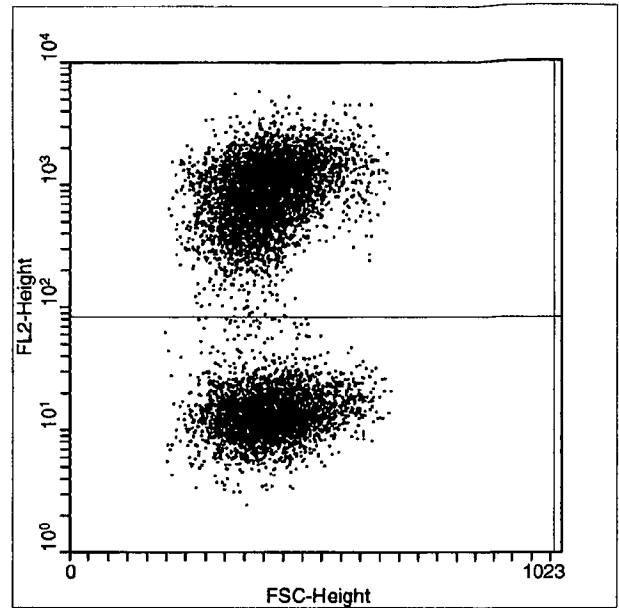
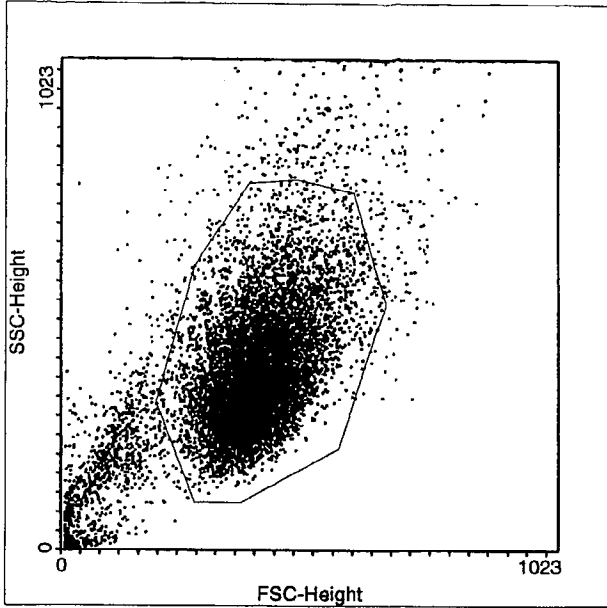
Quad Stats

FSC-Height(Lin) vs FL2-Height(Log)

Quadrant x,y: 1008,492

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	405.7	866.6	9912	49.56	56.01
2 UR	0.0	0.0	0	0.00	<del>0.00</del>
3 LL	409.6	13.3	7786	38.93	43.99
4 LR	0.0	0.0	0	0.00	0.00

> 1.27



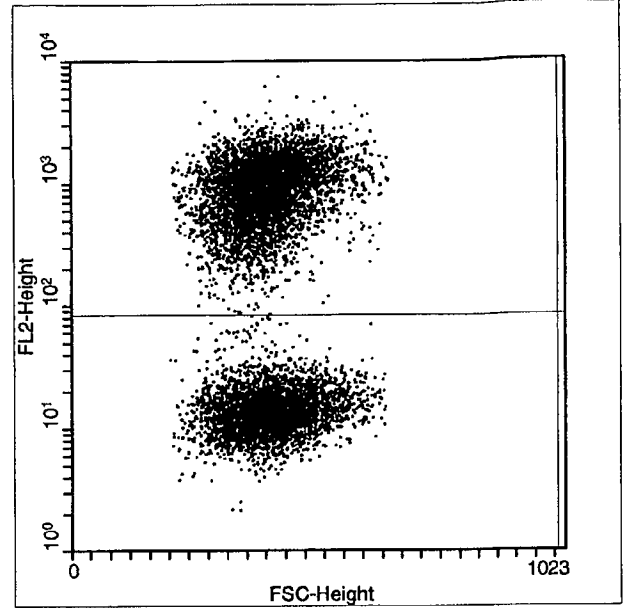
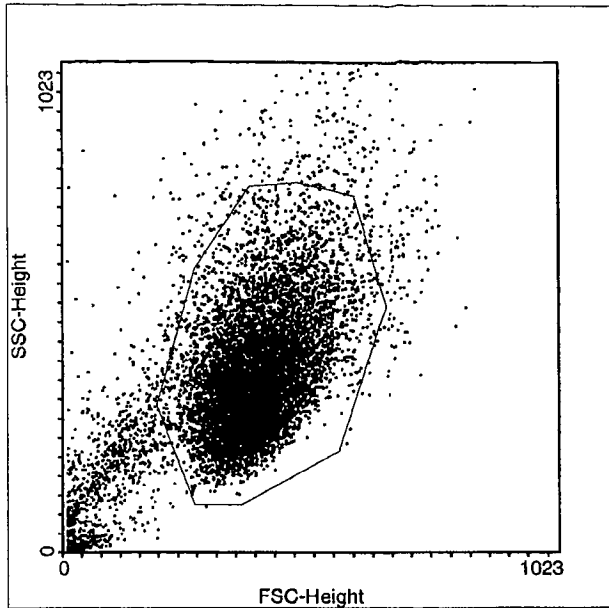
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 29 15:00:04 2002

Gates: R1  
 Project: Experiment: 0 0  
 File: 052902 Sample: 0Gy(DiI)+10Gy 22h  
 Date: 29-May-2 Parameters: 7  
 Total Events 20000 Gated Events 17143 85.71%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FSC-Height(Lin) vs FL2-Height(Log)  
 Quadrant x,y: 1008,492

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	406.9	881.6	10056	50.28	58.66
2 UR	0.0	0.0	0	0.00	0.00
3 LL	415.4	13.6	7087	35.44	41.34
4 LR	0.0	0.0	0	0.00	0.00

>1.42



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed May 29 15:00:49 2002  
 Gates: R1  
 Project: Experiment: 2  
 File: 052902 Sample: 0Gy(DII)+20Gy 22h  
 Date: 29-May-2 Parameters: /  
 Total Events 20000 Gated Events 17278 86.39%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FSC-Height(Lin) vs FL2-Height(Log)  
 Quadrant x,y: 1008,492

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	402.2	866.0	10004	50.02	57.90
2 UR	0.0	0.0	0	0.00	<del>0.00</del>
3 LL	409.7	13.6	7274	36.37	42.10
4 LR	0.0	0.0	0	0.00	0.00

1.38



MAY 31, 2002

CALCULATIONS (FCM)

$$\textcircled{1} \frac{0.6g(x_1) + 0.6g(y_1)}{0.6g(x_2) + 0.5g(y_2)} = \frac{50.9}{49.1} = 1.04$$

$$x_1(\underline{50\%}) + y_1(\underline{50\%}) = 100 \text{ (events)} \rightarrow$$

CONTROL
$\frac{0.6g}{0.6g} - \frac{50}{50}$

$$\frac{100}{0.6g(x_2) + 0.5g(y_2)} = 1.04$$

$$0.6g(x_2) + 0.5g(y_2) = 96.2$$

$$\text{CONVERTED } x_2 = \frac{96.2 \times 60.7\%}{100\%} = \underline{58.4 - 0.6g}$$

$$\text{CONVERTED } y_2 = \frac{96.2 \times 39.3}{100\%} = \underline{37.8 - 0.5g}$$

$$\textcircled{2} \frac{0.6g(x_1) + 1.0g(y_1)}{0.6g(x_2) + 1.0g(y_2)} = \frac{50.8}{49.2} = 1.03$$

$$\frac{100}{0.6g(x_2) + 1.0g(y_2)} = 1.03$$

$$0.6g(x_2) + 1.0g(y_2) = 97.1$$

$$\text{CONVERTED } x_2 = \frac{97.1 \times 65.3\%}{100\%} = \underline{63.4 - 0.6g}$$

$$\text{CONVERTED } y_2 = \frac{97.1 \times 34.7}{100\%} = \underline{33.7 - 1.0g}$$

$\textcircled{3}$  See over. ↓

$$(3) \frac{0Gy(x_1) + 0Gy(y_1)}{0Gy(x_2) + 5Gy(y_2)} = \frac{56.0}{44.0} = 1.27$$

$$\frac{100}{0Gy(x_2) + 5Gy(y_2)} = 1.27$$

$$0Gy(x_2) + 5Gy(y_2) = 78.7$$

$$\text{CONVERTED } X_2 = \frac{78.7 \times 77.2\%}{100\%} = 60.8 - 0Gy$$

$$\text{CONVERTED } Y_2 = \frac{78.7 \times 22.8\%}{100\%} = 17.9 - 5Gy$$

$$(4) \frac{0Gy(x_1) + 0Gy(y_1)}{0Gy(x_2) + 10Gy(y_2)} = \frac{58.7}{41.3} = 1.42$$

$$\frac{100}{0Gy(x_2) + 10Gy(y_2)} = 1.42$$

$$0Gy(x_2) + 10Gy(y_2) = 70.4$$

$$\text{CONVERTED } X_2 = \frac{70.4 \times 81.9\%}{100\%} = 57.7 - 0Gy$$

$$\text{CONVERTED } Y_2 = \frac{70.4 \times 18.1\%}{100\%} = 12.7 - 10Gy$$

$$(5) \frac{0Gy(x_1) + 0Gy(y_1)}{0Gy(x_2) + 20Gy(y_2)} = \frac{57.9}{42.1} = 1.38$$

$$\frac{100}{0Gy(x_2) + 20Gy(y_2)} = 1.38$$

$$0Gy(x_2) + 20Gy(y_2) = 72.5$$

$$\text{CONVERTED } X_2 = \frac{72.5 \times 88.4\%}{100\%} = 64.1 - 0Gy$$

$$\text{CONVERTED } Y_2 = \frac{72.5 \times 11.6\%}{100\%} = 8.4 - 20Gy$$

#1

Bystander Studies  
⑧

June 1, 2002

BYSTANDER EFFECTS (1 - IRRADIATION)

WB / 20 100% confluency

0 Gy — For counting of plated cells (hemacytometer)

0 Gy

0 Gy — DiI (40 min)

0.5 Gy — 0.14 min

1.0 Gy — 0.29 min

5.0 Gy — 1.45 min

10.0 Gy — 2.89 min

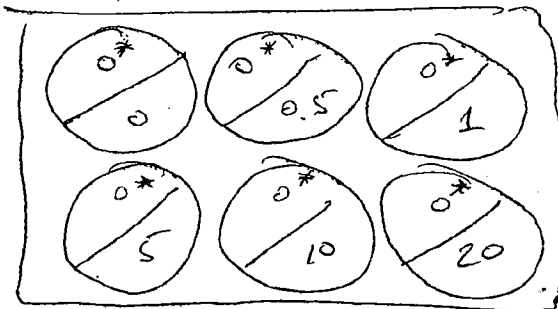
20.0 Gy — 5.79 min

100µL / 100µL — SCENE

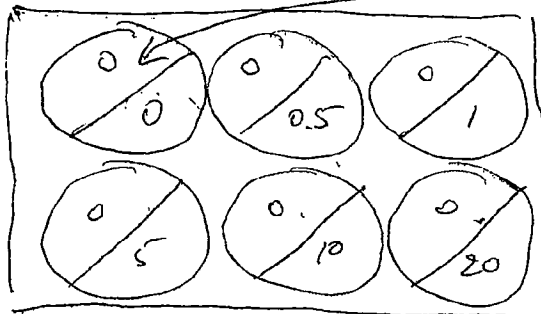
IRRADIATION TIME: 9:30

ONE SOURCE: 345.59 Rad/min.

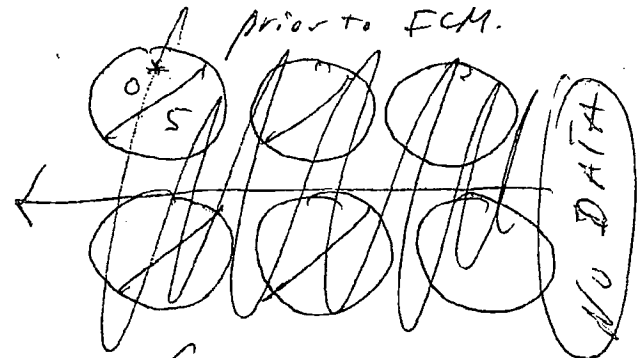
PLATING TIME: 11:00



Note: 0\* - 0 Gy - DiI - stained

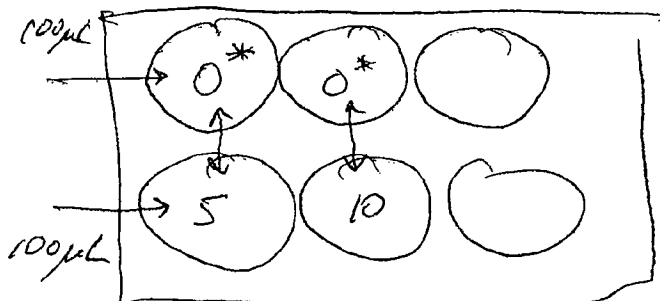


To be stained with DiI prior to FCM.



~~Counter Counting~~

~~To be combined prior to FCM.~~



0 Gy → 1.5 mL

496 } 1040 × 10<sup>3</sup> cells/mL × 2  
544 } 2080 × 10<sup>3</sup> cells/mL

dilution factor  
↓  
x 2

HEMOCYTOMETER COUNTING: 3.12 × 10<sup>6</sup> cells in 1.5 mL OR  
in 5 cm disk (Ø 5)

208 × 10<sup>3</sup> cells in 100 μL (FOR PLATING)

100 μL + 100 μL → 416 × 10<sup>3</sup> cells

JUNE 2, 2002

HEMOCYTOMETER COUNTING:

\* 0 Gy / 0 Gy: 243 } 513 × 10<sup>3</sup> cells/mL  
270 } + 0.5 mL → 770 × 10<sup>3</sup> cells

\* 0 Gy / 0.5 Gy: 221 } 453 × 10<sup>3</sup> cells/mL  
232 } + 0.5 mL → 680 × 10<sup>3</sup> cells

\* 0 Gy / 1 Gy: 210 } 416 × 10<sup>3</sup> cells/mL → ~~570~~  
206 } + 0.5 mL → 624 × 10<sup>3</sup> cells

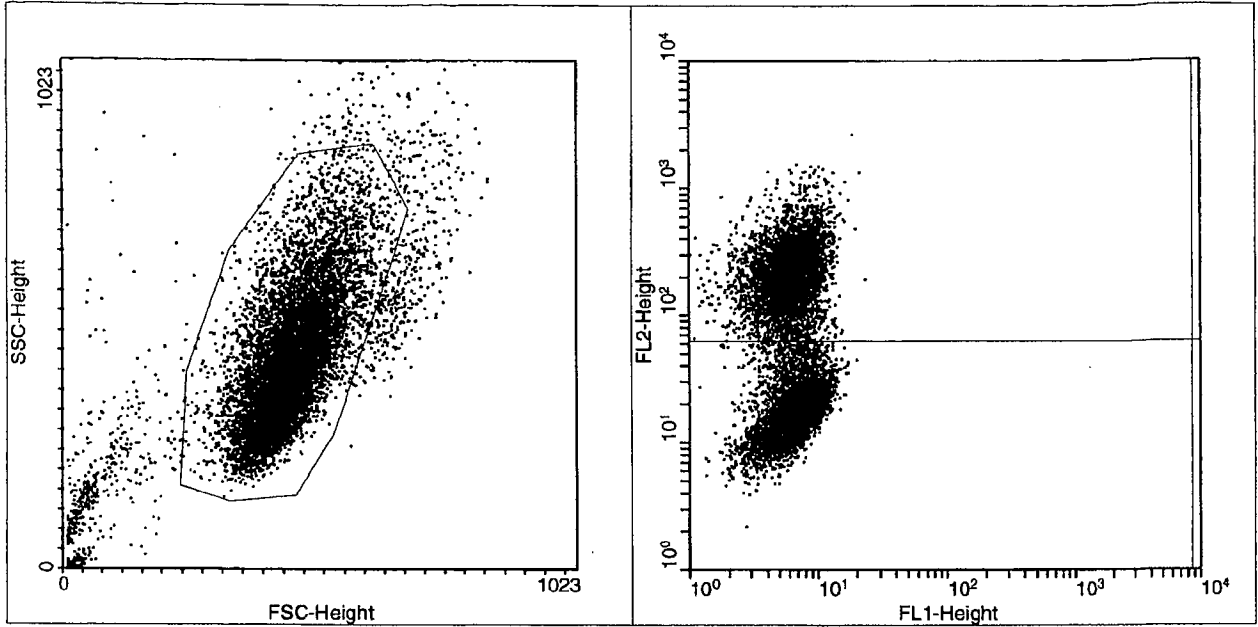
\* 0 Gy / 5 Gy: 170 } 330 × 10<sup>3</sup> cells/mL  
160 } + 0.5 mL → 495 × 10<sup>3</sup> cells

\* 0 Gy / 10 Gy: 194 } 394 × 10<sup>3</sup> cells/mL  
200 } + 0.5 mL → 591 × 10<sup>3</sup> cells

\* 0 Gy / 20 Gy: 140 } 283 × 10<sup>3</sup> cells/mL  
143 } + 0.5 mL → 425 × 10<sup>3</sup> cells

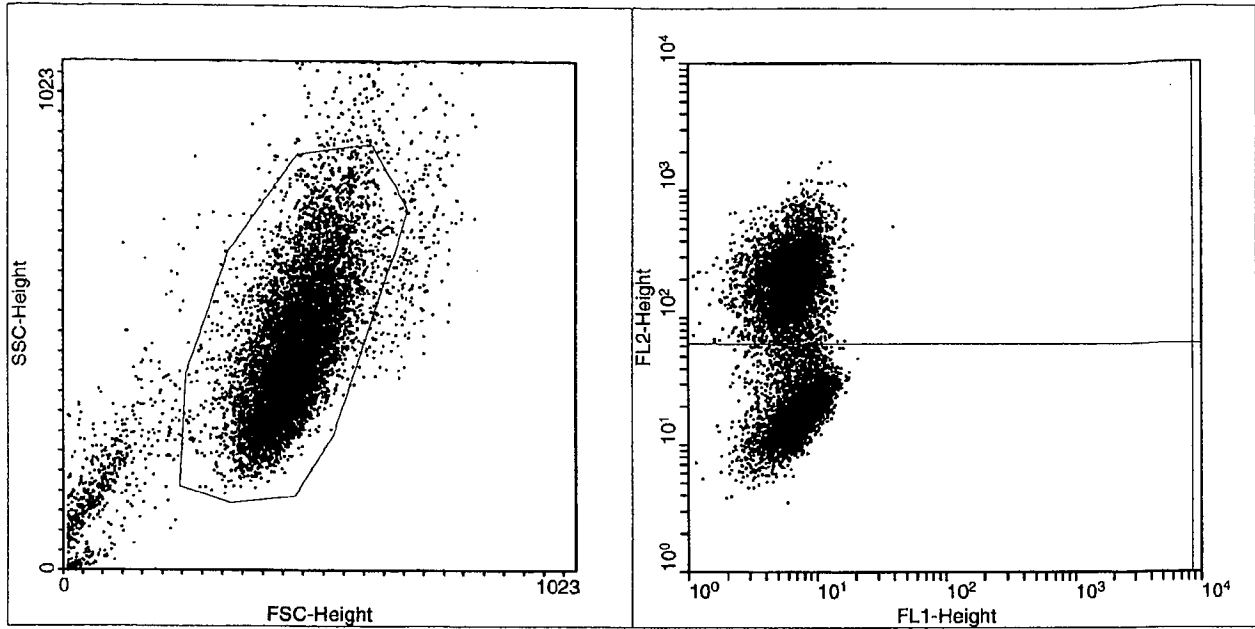
\* 0 Gy + 5 Gy: 183 } 359 × 10<sup>3</sup> cells/mL  
176 } + 0.5 mL → 539 × 10<sup>3</sup> cells

\* 0 Gy + 10 Gy: 117 } 275 × 10<sup>3</sup> cells/mL  
158 } + 0.5 mL → 413 × 10<sup>3</sup> cells



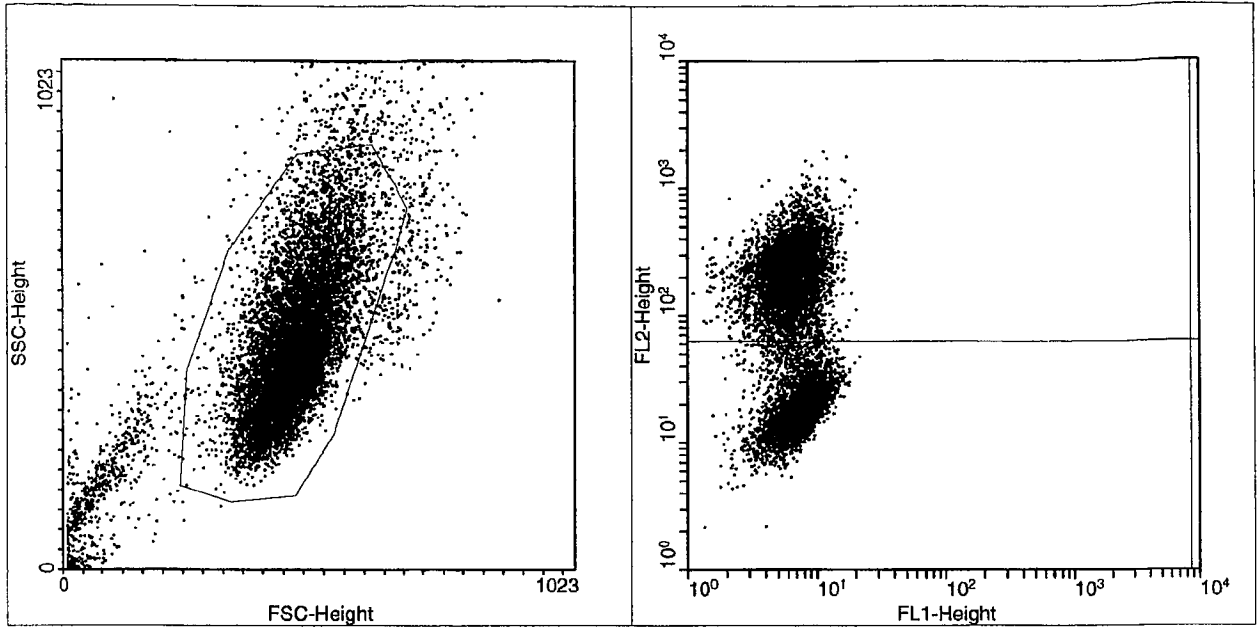
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jun 03 10:59:27 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060302 Sample: 0Gy(DiI)/0Gy  
 Date: 3-Jun-2 Parameters: 7  
 Total Events 20000 Gated Events 18001 90.00%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,460

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	6.1	213.4	8743	43.72	48.57
2 UR	0.0	0.0	0	0.00	0.00
3 LL	6.5	17.4	9258	46.29	51.43
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jun 03 11:03:20 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060302 Sample: 0Gy (DI1) / 0.5Gy  
 Date: 3-Jun-2 Parameters: 7  
 Total Events 20000 Gated Events 17967 89.83%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,460

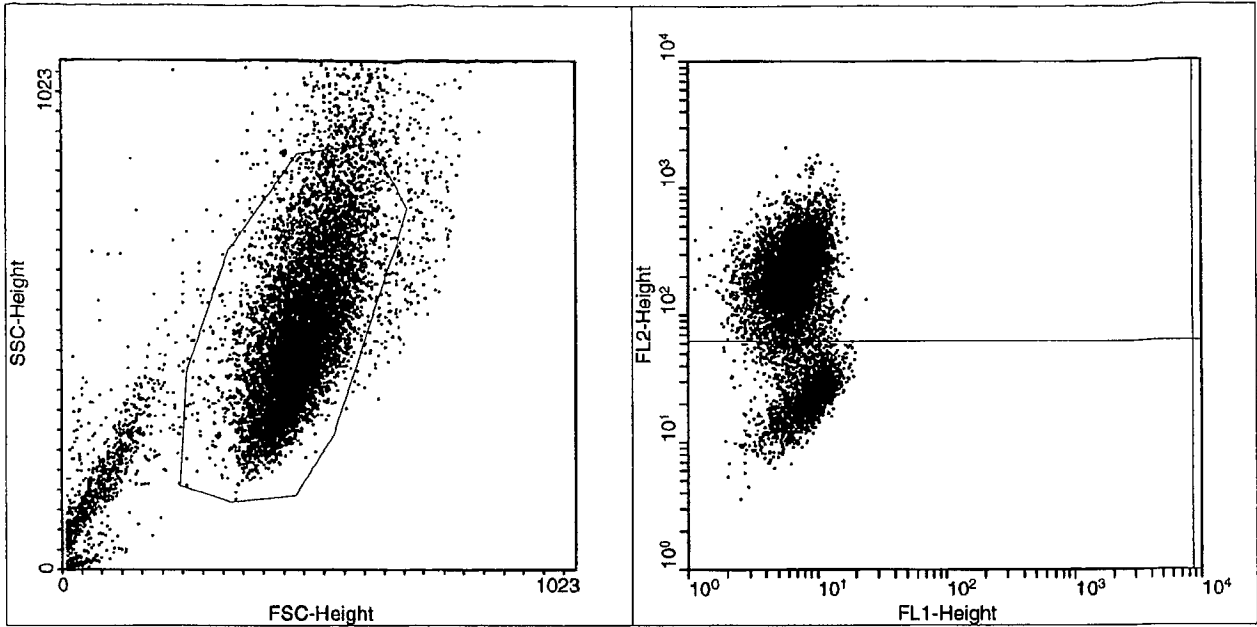
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	6.3	211.0	9765	48.83	54.35
2 UR	0.0	0.0	0	0.00	0.00
3 LL	6.9	18.7	8202	41.01	45.65
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jun 03 11:04:07 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060302 Sample: 0Gy(DiI)/1Gy  
 Date: 3-Jun-2 Parameters: 7  
 Total Events 20000 Gated Events 17447 87.24%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,460

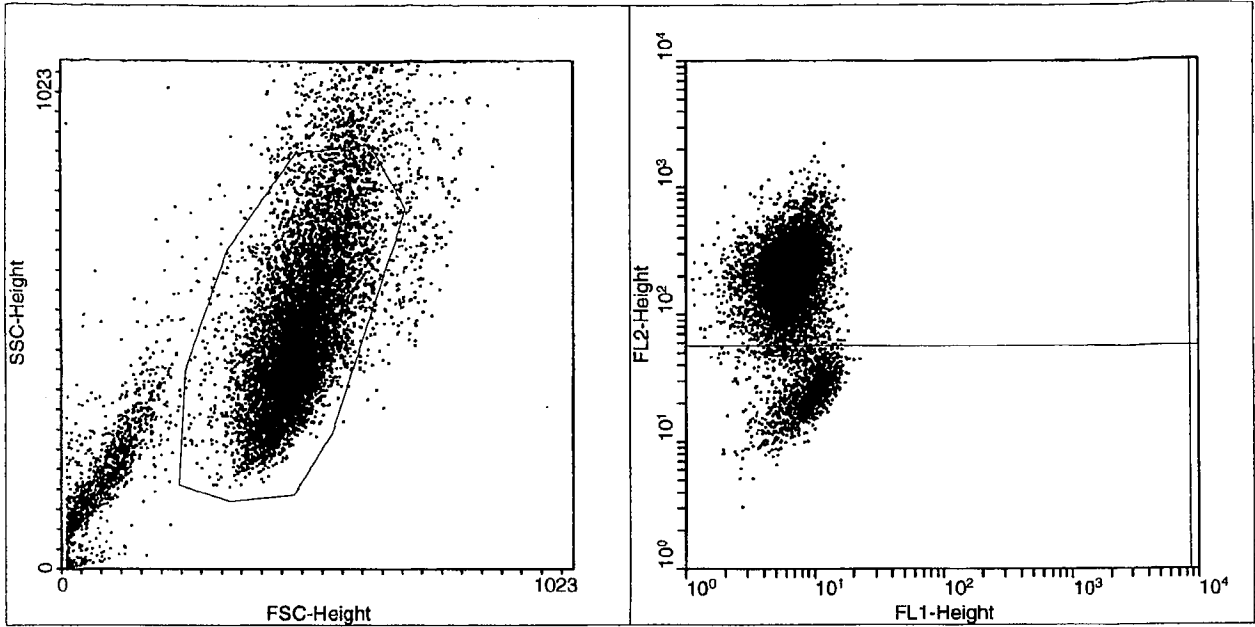
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	6.5	215.3	10338	51.69	59.25
2 UR	0.0	0.0	0	0.00	0.00
3 LL	7.3	19.3	7109	35.55	40.75
4 LR	0.0	0.0	0	0.00	0.00





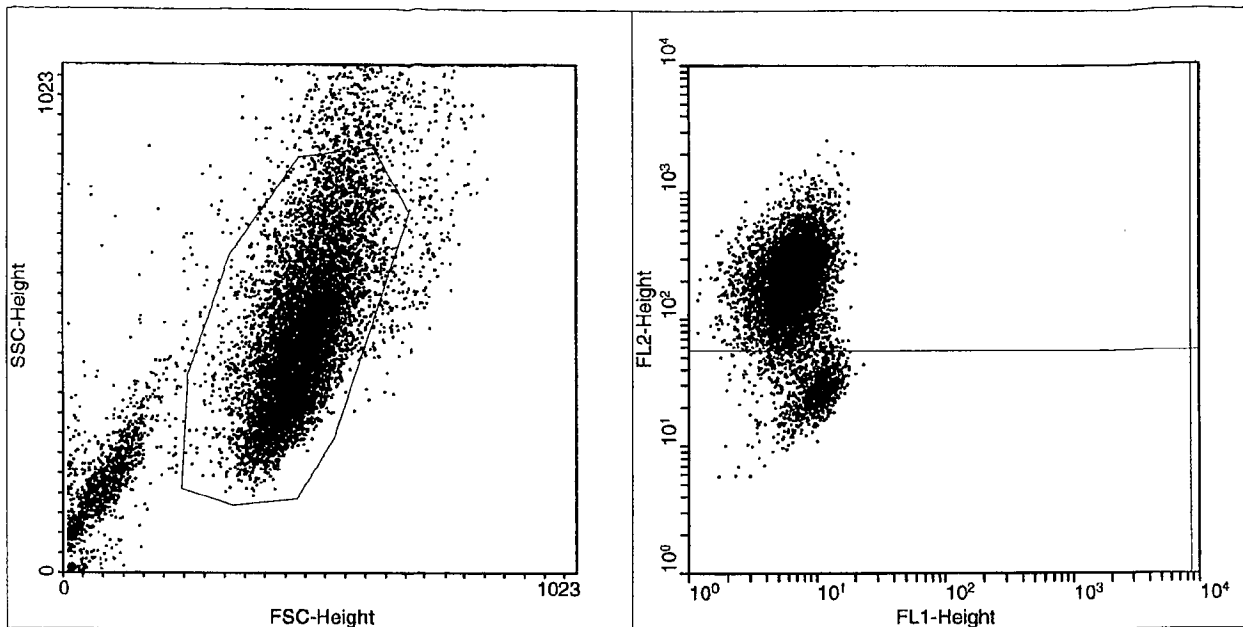
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jun 03 11:05:14 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060302 Sample: 0Gy(DiI)/5Gy  
 Date: 3-Jun-2 Parameters: 7  
 Total Events 20000 Gated Events 16727 83.64%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,460

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	6.5	215.9	12421	62.11	74.26
2 UR	0.0	0.0	0	0.00	0.00
3 LL	8.0	24.2	4306	21.53	25.74
4 LR	0.0	0.0	0	0.00	0.00



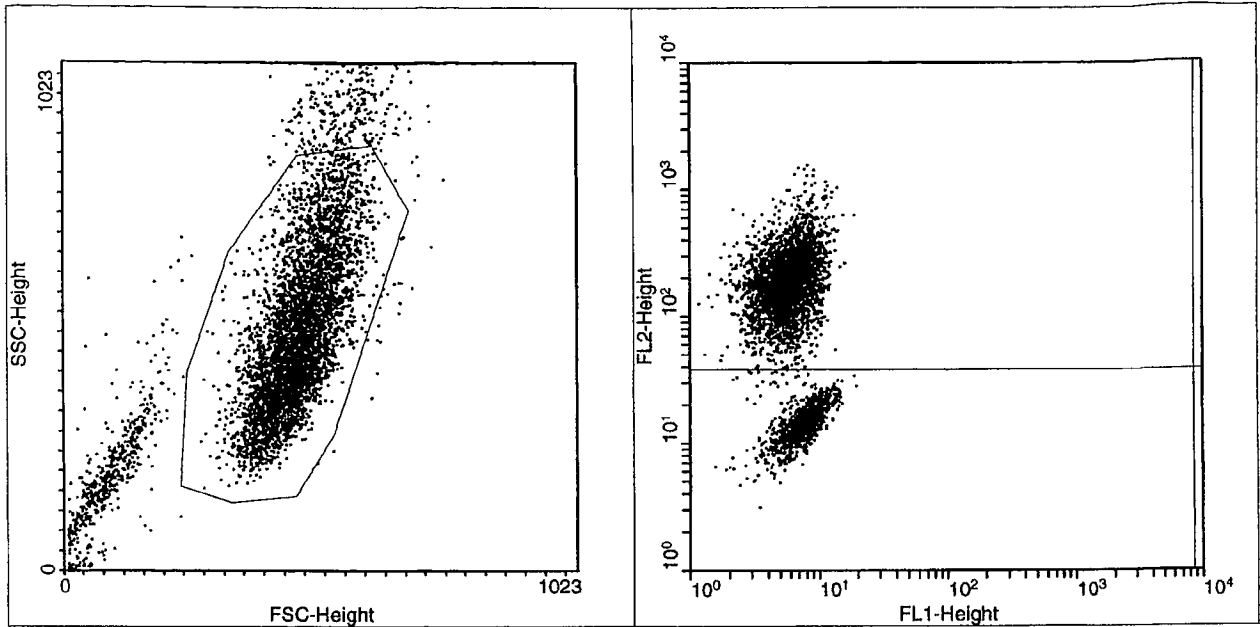
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jun 03 11:13:29 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060302 Sample: 0Gy (DiI) / 10Gy  
 Date: 3-Jun-2 Parameters: 7  
 Total Events 20000 Gated Events 15788 78.94%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,448

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	6.6	214.3	13041	65.21	82.60
2 UR	0.0	0.0	0	0.00	0.00
3 LL	8.3	25.1	2747	13.74	17.40
4 LR	0.0	0.0	0	0.00	0.00



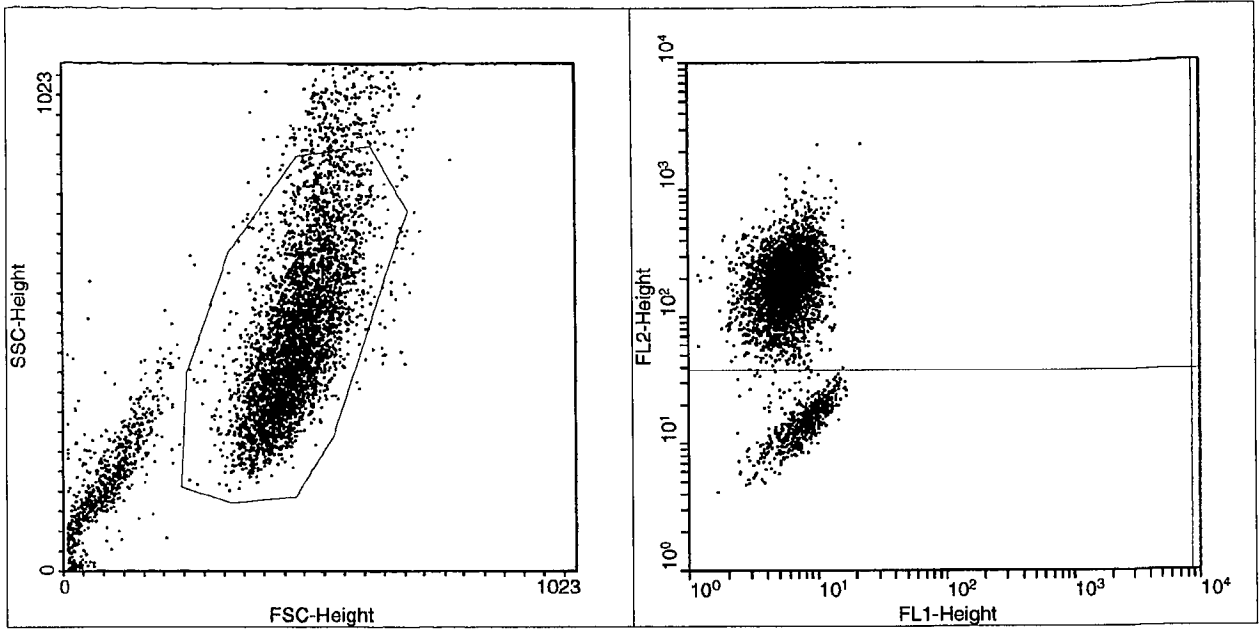
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jun 03 11:13:59 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060302 Sample: 0Gy (DiI) / 20Gy  
 Date: 3-Jun-2 Parameters: 7  
 Total Events 20000 Gated Events 15854 79.27%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,448

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	6.5	213.3	13775	68.88	<u>86.89</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	8.7	28.1	2079	10.40	<u>13.11</u>
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jun 03 11:16:54 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060302 Sample: 0Gy(DiI)+5Gy  
 Date: 3-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 7995 79.95%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,404

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.8	188.8	6011	60.11	75.18
2 UR	0.0	0.0	0	0.00	0.00
3 LL	7.5	14.4	1984	19.84	24.82
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jun 03 11:17:28 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060302 Sample: 0Gy (DiI)+10Gy  
 Date: 3-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 7497 74.97%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,404

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.8	188.4	6348	63.48	<u>84.67</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	7.6	14.8	1149	11.49	<u>15.33</u>
4 LR	0.0	0.0	0	0.00	0.00

JUNE 3, 2002

①

CALCULATIONS (AMOUNTS OF UNIRRADIATED CELLS IN THE CO-CULTURES WITH IRRADIATED CELLS)

0 Gy / 0 Gy

$$\frac{270 \times 10^3}{x} = \frac{100\%}{48.6\%}$$

$$x = \frac{270 \times 10^3 \times 48.6\%}{100\%} = 374 \times 10^3 \text{ cells}$$

0 Gy\* / 0.5 Gy

$$\frac{680 \times 10^3 \times 54.4\%}{100\%} = 370 \times 10^3 \text{ cells}$$

0 Gy\* / 1 Gy

$$\frac{624 \times 10^3 \times 59.3\%}{100\%} = 370 \times 10^3 \text{ cells}$$

0 Gy\* / 5 Gy

$$\frac{495 \times 10^3 \times 74.3\%}{100\%} = 368 \times 10^3 \text{ cells}$$

0 Gy\* / 10 Gy

$$\frac{591 \times 10^3 \times 82.6\%}{100\%} = 488 \times 10^3 \text{ cells} - ?$$

0 Gy\* / 20 Gy

$$\frac{425 \times 10^3 \times 86.9\%}{100\%} = 369 \times 10^3 \text{ cells}$$

②

AMOUNTS OF IRRADIATED CELLS IN THE CO-CULTURES WITH UNIRRADIATED CELLS

0 Gy / 0 Gy

$$\frac{770 \times 10^3}{x} = \frac{100\%}{51.4\%}$$

$$x = \frac{770 \times 51.4 \times 10^3}{100\%} = 396 \times 10^3 \text{ cells}$$

0 Gy\* / 0.5 Gy

$$\frac{680 \times 10^3 \times 45.7\%}{100\%} = 311 \times 10^3 \text{ cells}$$

0 Gy\* / 1 Gy

$$\frac{624 \times 10^3 \times 40.8\%}{100\%} = 255 \times 10^3 \text{ cells}$$

0 Gy\* / 5 Gy

$$\frac{495 \times 10^3 \times 25.7\%}{100\%} = 127 \times 10^3 \text{ cells}$$

0 Gy\* / 10 Gy

$$\frac{591 \times 10^3 \times 17.4\%}{100\%} = 103 \times 10^3 \text{ cells}$$

0 Gy\* / 20 Gy

$$\frac{425 \times 10^3 \times 13.1\%}{100\%} = 56 \times 10^3 \text{ cells}$$

(3) AMOUNTS OF UNIRRADIATED AND IRRADIATED CELLS  
CULTURED SEPARATELY

$$\textcircled{0} \text{ Gy}^* + 5 \text{ Gy} \quad \frac{539 \times 10^3 \times 75.2\%}{100\%} = \textcircled{405 \times 10^3 \text{ cells}}$$

$$\textcircled{0} \text{ Gy}^* + \textcircled{5} \text{ Gy} \quad \frac{539 \times 10^3 \times 24.8\%}{100\%} = \textcircled{134 \times 10^3 \text{ cells}}$$

$$\textcircled{0} \text{ Gy}^* + 10 \text{ Gy} \quad \frac{413 \times 10^3 \times 84.7\%}{100\%} = \textcircled{350 \times 10^3 \text{ cells}}$$

$$\textcircled{0} \text{ Gy}^* + \textcircled{10} \text{ Gy} \quad \frac{413 \times 10^3 \times 15.3\%}{100\%} = \textcircled{63 \times 10^3 \text{ cells}}$$

#2



June 5, 2002

BYSTANDER EFFECTS (β - IRRADIATION)

WB/21 100% Confluency

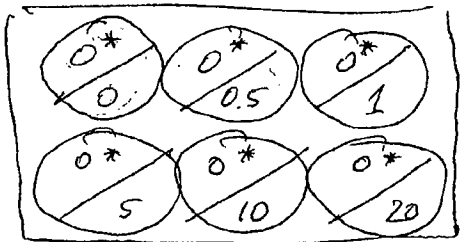
- 0 Gy
- 0 Gy
- 0 Gy For DiI staining (40 min)
- 0.5 Gy - 0.14 mls
- 1.0 Gy - 0.29 mls
- 5.0 Gy - 1.45 mls
- 10.0 Gy - 2.89 mls
- 20.0 Gy - 5.79 mls

IRRADIATION TIME: 8:20

ONE SOURCE: 345.59 Rad/mls

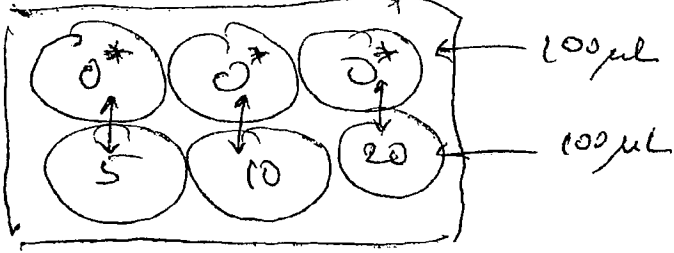
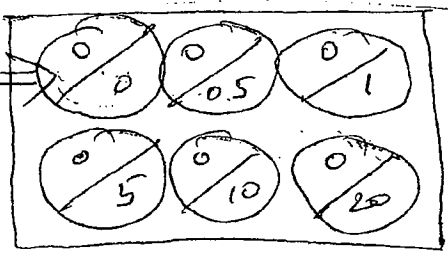
PLATING TIME: 10:00

100 μL / 100 μL - SCHEME



Note: 0\* - 0 Gy - DiI stained

To be stained prior to FCM



0 Gy

1.5 mL

551  
582

$1133 \times 10^3$  cells/mL  $\times 2$

dilution factor

$2266 \times 10^3$  cells/mL

5 cm dish or 1.5 mL  $\leftarrow 3.39 \times 10^6$  cells.

PLATED: 100  $\mu$ L containing  $227 \times 10^3$  cells.

OR 100  $\mu$ L + 100  $\mu$ L  $\rightarrow 454 \times 10^3$  cells.

JUNE 6, 2002

HEMOCYTOMETER COUNTING:

0 Gy\* / 0 Gy:

224 } 448  
224 }  
243 } 442  
199 }

$\frac{890}{2} = 445 \times 10^3$  cells/mL

+ 0.5 mL  $\rightarrow 668 \times 10^3$  cells

0 Gy\* / 0.5 Gy:

232 } 382  
150 }  
186 } 368  
182 }

$\frac{750}{2} = 375 \times 10^3$  cells/mL

+ 0.5 mL  $\rightarrow 563 \times 10^3$  cells

0 Gy\* / 1 Gy:

240 } 467  
227 }  
209 } 427  
218 }

$\frac{894}{2} = 447 \times 10^3$  cells/mL

+ 0.5 mL  $\rightarrow 671 \times 10^3$  cells

0 Gy\* / 5 Gy:

176 } 358  
182 }  
162 } 356  
194 }

$\frac{714}{2} = 357 \times 10^3$  cells/mL

+ 0.5 mL  $\rightarrow 534 \times 10^3$  cells

0 Gy\* / 10 Gy:

138 } 276  
138 }  
134 } 271  
137 }

$\frac{547}{2} = 274 \times 10^3$  cells/mL

+ 0.5 mL  $\rightarrow 411 \times 10^3$  cells

0 Gy\* / 20 Gy:

130 } 263  
133 }  
136 } 282  
146 }

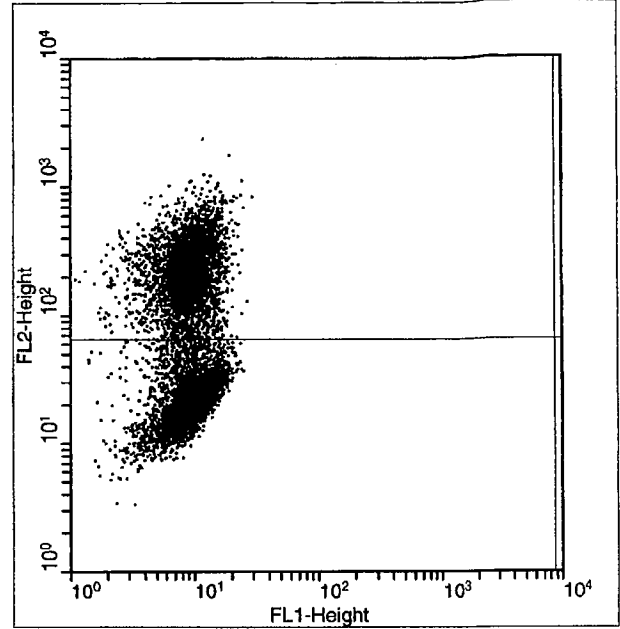
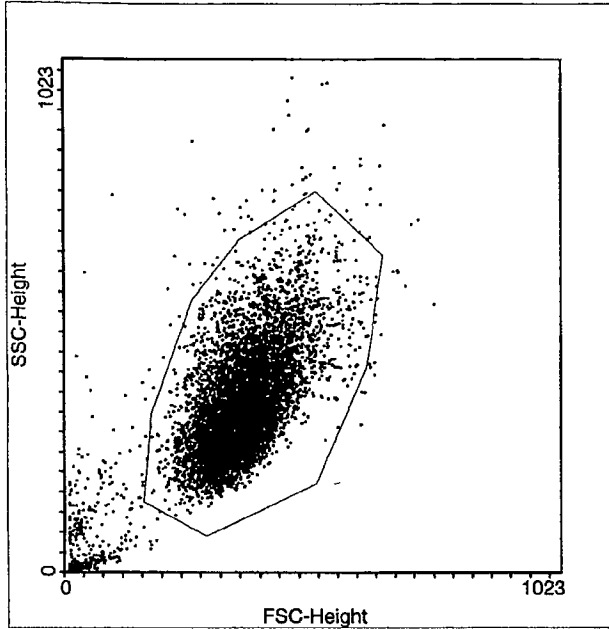
$\frac{545}{2} = 273 \times 10^3$  cells/mL

+ 0.5 mL  $\rightarrow 409 \times 10^3$  cells

$$0Gy^* + 5Gy: \begin{array}{l} 130 \\ 136 \\ 137 \\ 129 \end{array} \left. \vphantom{\begin{array}{l} 130 \\ 136 \\ 137 \\ 129 \end{array}} \right\} \begin{array}{l} 266 \\ 266 \end{array} \left. \vphantom{\begin{array}{l} 266 \\ 266 \end{array}} \right\} 266 \times 10^3 \text{ cells/mL} \\ + 0.5 \text{ mL} \rightarrow 399 \times 10^3 \text{ cells}$$

$$0Gy^* + 10Gy: \begin{array}{l} 118 \\ 132 \\ 130 \\ 142 \end{array} \left. \vphantom{\begin{array}{l} 118 \\ 132 \\ 130 \\ 142 \end{array}} \right\} \begin{array}{l} 250 \\ 272 \end{array} \left. \vphantom{\begin{array}{l} 250 \\ 272 \end{array}} \right\} \frac{522}{2} = 261 \times 10^3 \text{ cells/mL} \\ + 0.5 \text{ mL} \rightarrow 392 \times 10^3 \text{ cells}$$

$$0Gy^* + 20Gy: \begin{array}{l} 100 \\ 101 \\ 114 \\ 102 \end{array} \left. \vphantom{\begin{array}{l} 100 \\ 101 \\ 114 \\ 102 \end{array}} \right\} \begin{array}{l} 201 \\ 216 \end{array} \left. \vphantom{\begin{array}{l} 201 \\ 216 \end{array}} \right\} \frac{417}{2} = 209 \times 10^3 \text{ cells/mL} \\ + 0.5 \text{ mL} \rightarrow 313 \times 10^3 \text{ cells}$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:29:06 2002

Gates: R1

Project: Experiment:

File: 060602 Sample: 0Gy (DiI) / 0Gy

Date: 6-Jun-2 Parameters: 7

Total Events 10000 Gated Events 9351 93.51%

System: Log Parameter Means: Geometric

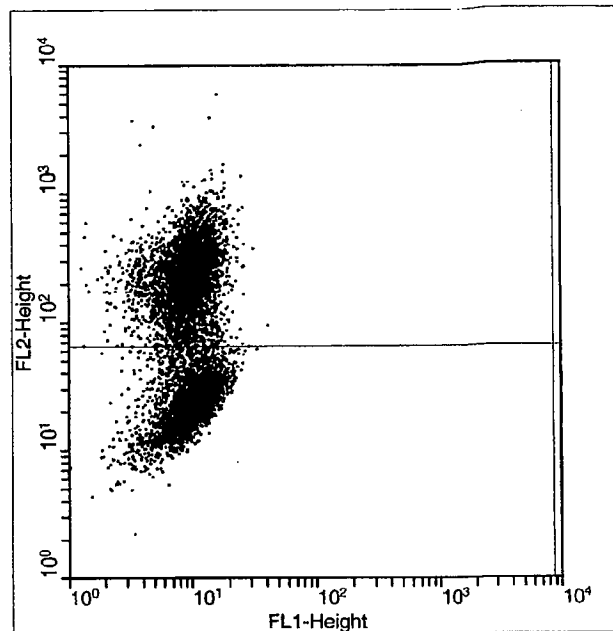
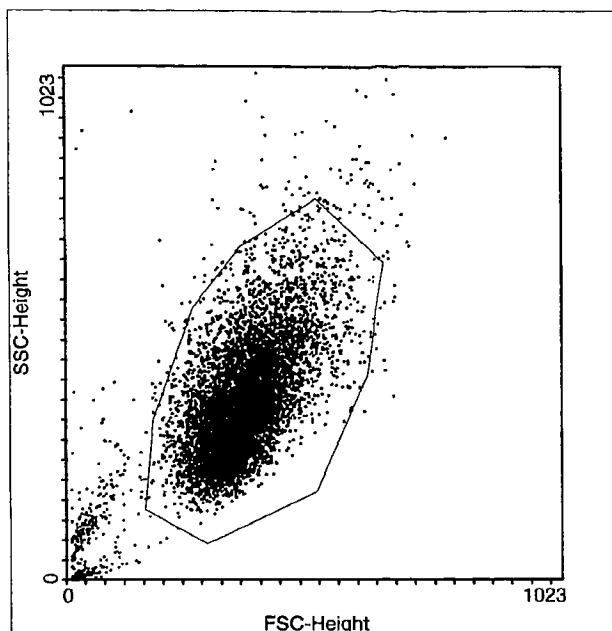
Quad Stats

FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 1008,464

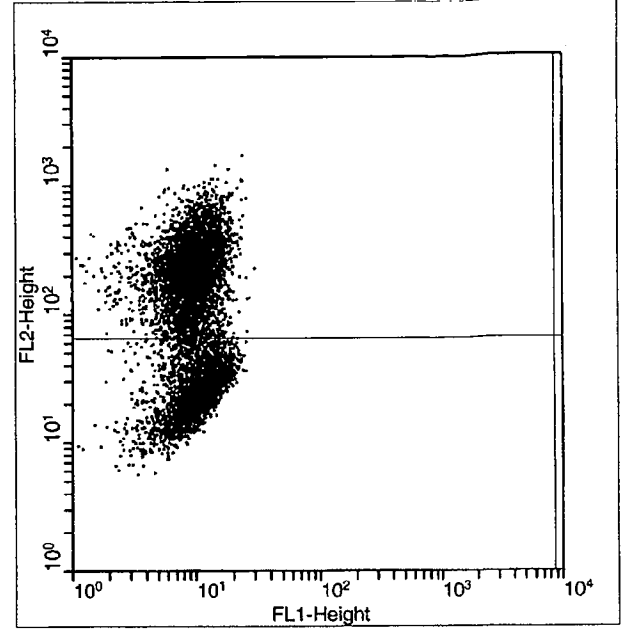
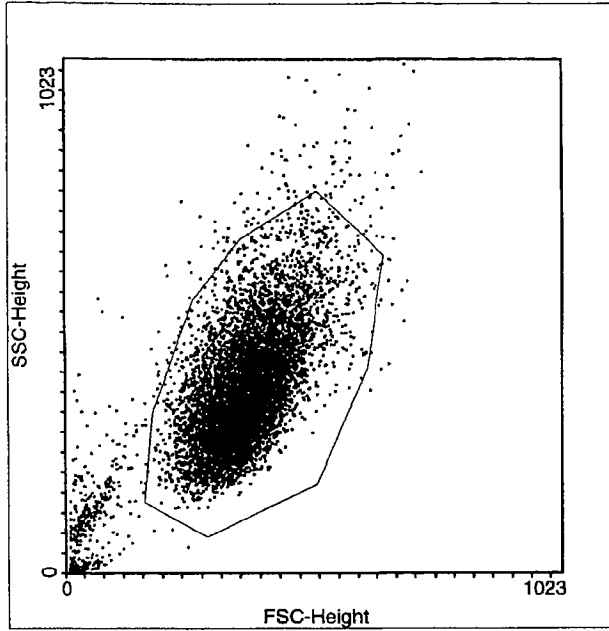
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	9.0	219.0	4616	46.16	49.36
2 UR	0.0	0.0	0	0.00	0.00
3 LL	9.0	21.7	4735	47.35	50.64
4 LR	0.0	0.0	0	0.00	0.00

= 0.97



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:32:12 2002  
 Gates: R1  
 Project: Experiment  
 File: 060002 Sample: 0Gy (Di1) / 0.5Gy  
 Date: 6-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9389 93.89%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,464

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	9.2	223.9	5186	51.86	55.23
2 UR	0.0	0.0	0	0.00	0.00
3 LL	9.7	23.3	4203	42.03	44.77
4 LR	0.0	0.0	0	0.00	0.00

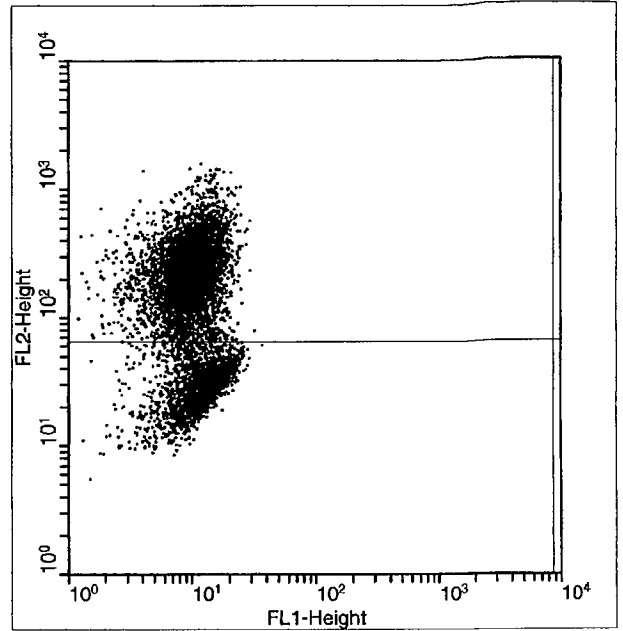
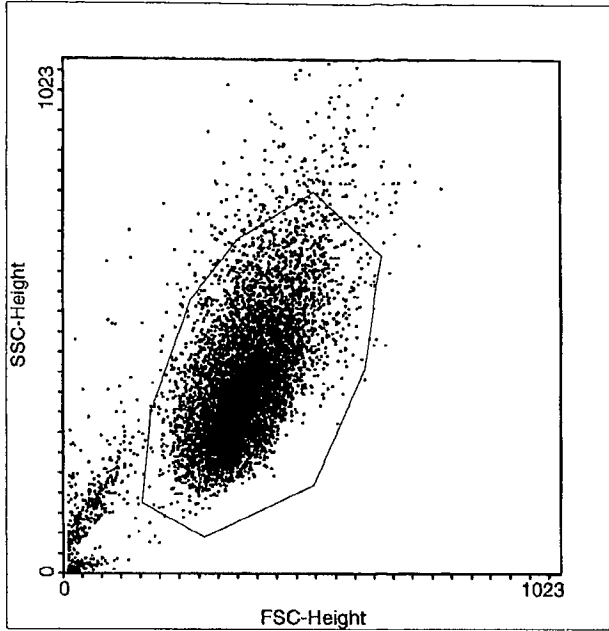


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:34:00 2002

Gates: R1  
 Project: Experiment:  
 File: 060602 Sample: Ogy(DiI)/1Gy  
 Date: 6-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9272 92.72%  
 System: Log Parameter Means: Geometric

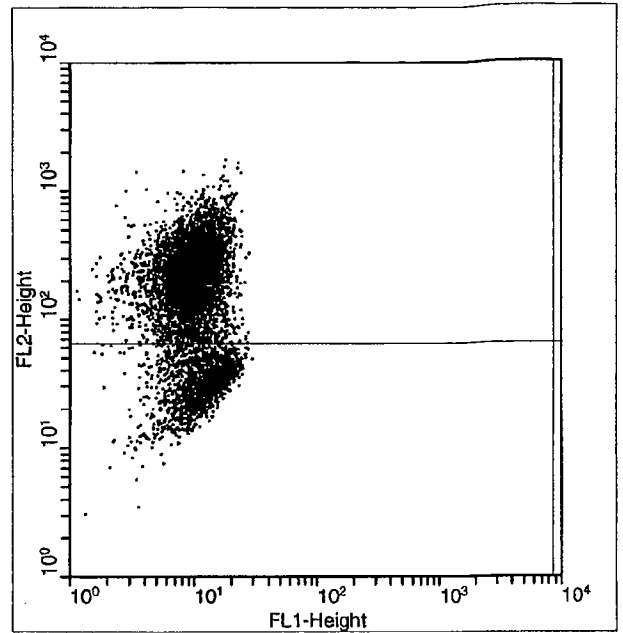
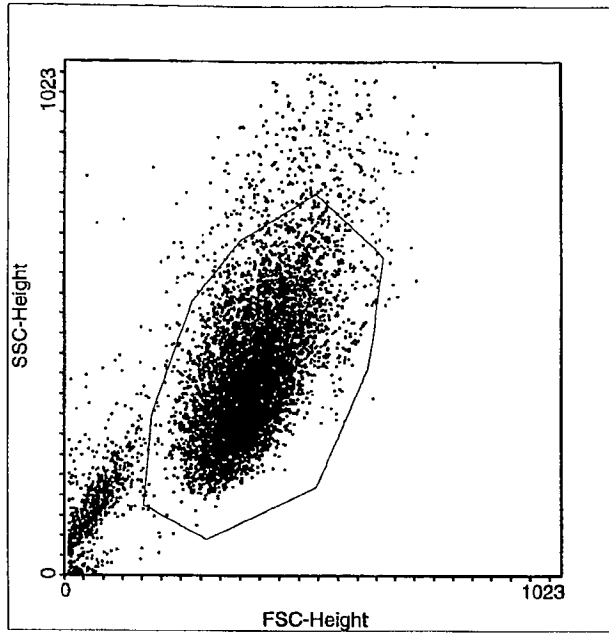
Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,464

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	9.2	217.4	5487	54.87	<u>59.18</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	9.9	25.2	3785	37.85	<u>40.82</u>
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:35:10 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060002 Sample: (0Gy(DiI)/5Gy)  
 Date: 6-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9079 90.79%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,464  

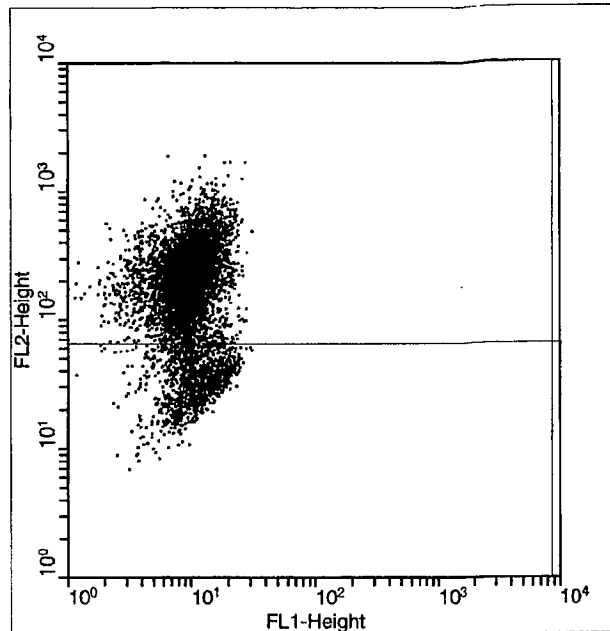
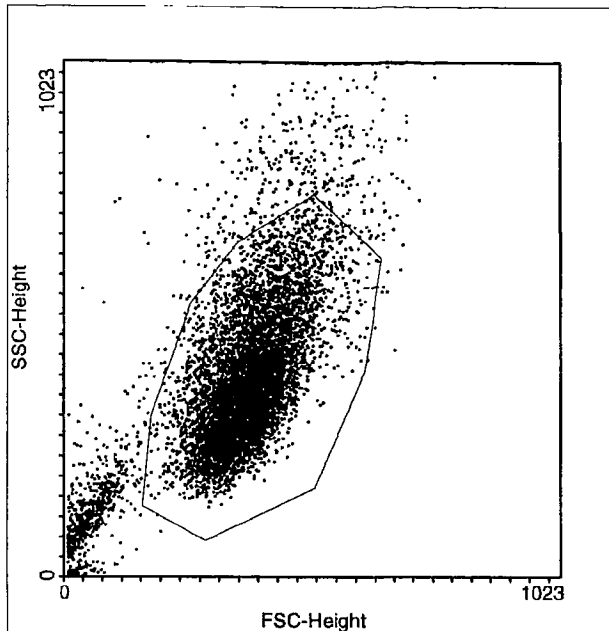
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	9.6	225.5	6428	64.28	70.80
2 UR	0.0	0.0	0	0.00	0.00
3 LL	11.0	29.2	2651	26.51	29.20
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:36:19 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060602 Sample: 0Gy(DiI)/10Gy  
 Date: 6-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8504 85.04%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,464  

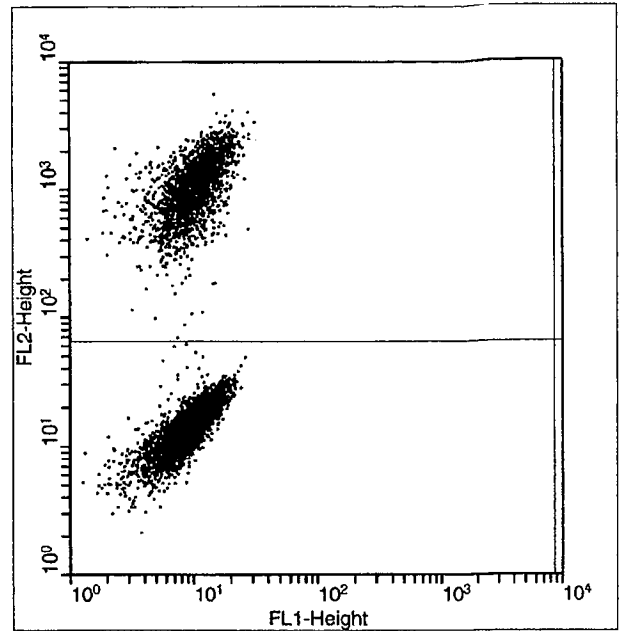
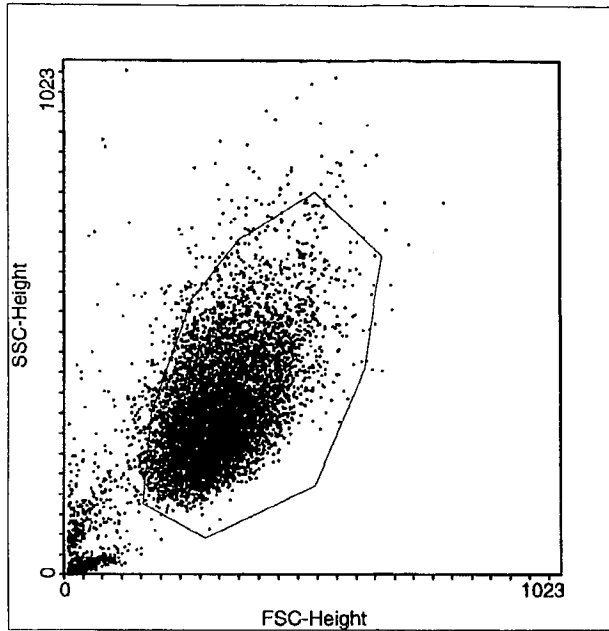
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	9.6	227.0	6449	64.49	<u>75.83</u>
2 UR	0.0	0.0	0	0.00	<u>0.00</u>
3 LL	11.1	31.6	2055	20.55	<u>24.17</u>
4 LR	0.0	0.0	0	0.00	<u>0.00</u>





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:37:26 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060002 Sample: QGY(DiI)/20Gy  
 Date: 6-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8538 85.38%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,464  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	9.5	225.9	6987	69.87	<u>81.83</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	10.8	34.3	1551	15.51	<u>18.17</u>
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:40:50 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060602 Sample: 0Gy/0Gy (DiI) + 0Gy/0.5Gy  
 Date: 6-Jun-2 Parameters: 1  
 Total Events 10000 Gated Events 8720 87.20%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,464

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	10.2	1022.9	2893	28.93	33.18
2 UR	0.0	0.0	0	0.00	0.00
3 LL	8.9	13.7	5827	58.27	66.82
4 LR	0.0	0.0	0	0.00	0.00

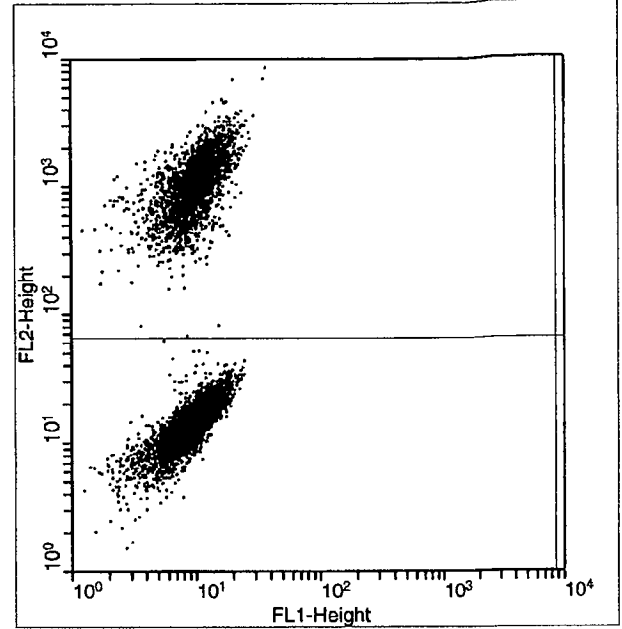
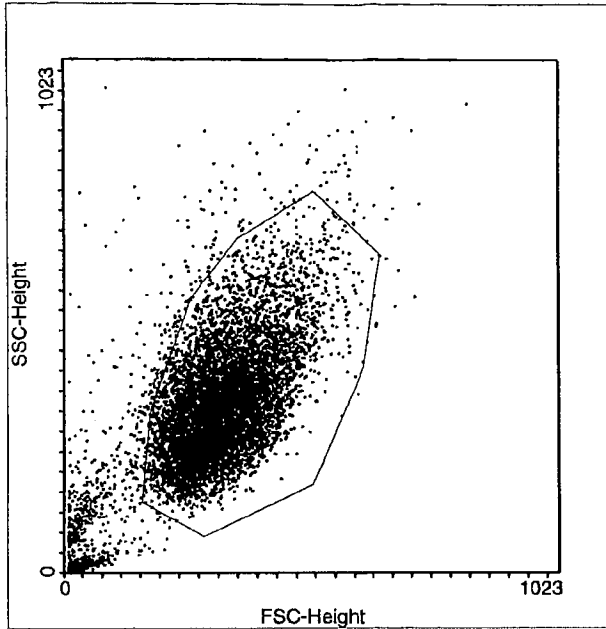
$$33.18 \times 2 = 66.36 = 0.99$$

$$66.36 + 66.82 = 133.18$$

$$133.18 - 100 = 33.18$$

$$66.36 - x$$

$$x = \frac{66.36 \times 100}{133.18} = \frac{49.83}{50.17}$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:46:51 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060002 Sample: 0Gy/0Gy (DiI)+0Gy/1Gy  
 Date: 6-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8745 87.45%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,464

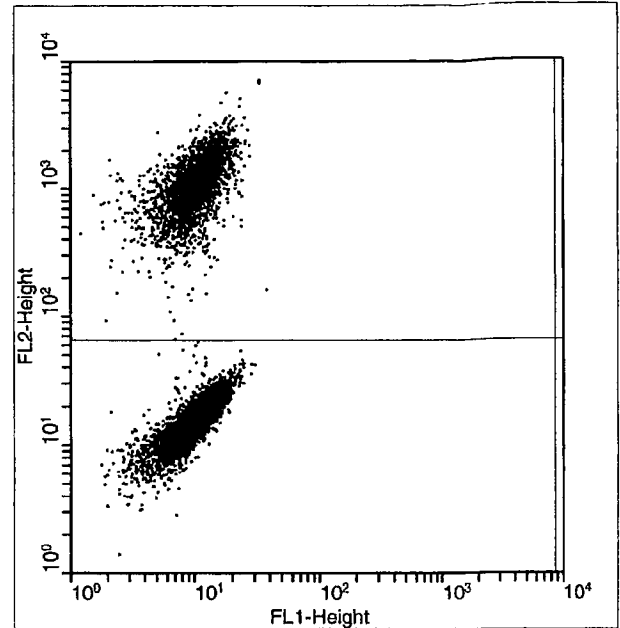
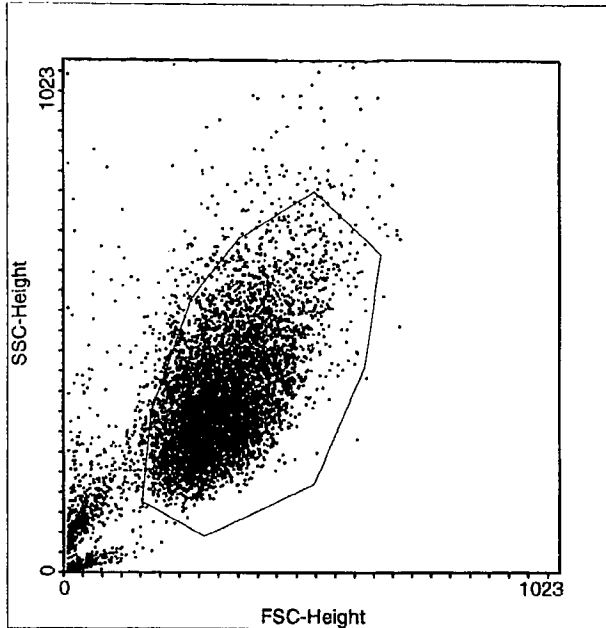
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	10.0	1025.9	3071	30.71	35.12
2 UR	0.0	0.0	0	0.00	0.00
3 LL	9.0	13.8	5674	56.74	64.88
4 LR	0.0	0.0	0	0.00	0.00

$35.12 \times 2 = 70.24 = 1.08$

$70.24 + 64.88 = 135.12$

$135.12 - 100\%$   
 $70.24 - x$

$x = \frac{70.24 \times 100}{135.12} = \frac{51.98}{48.02}$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:49:10 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060202 Sample: 0Gy/0Gy (DiI)+0Gy/5Gy  
 Date: 6-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8469 84.69%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,464

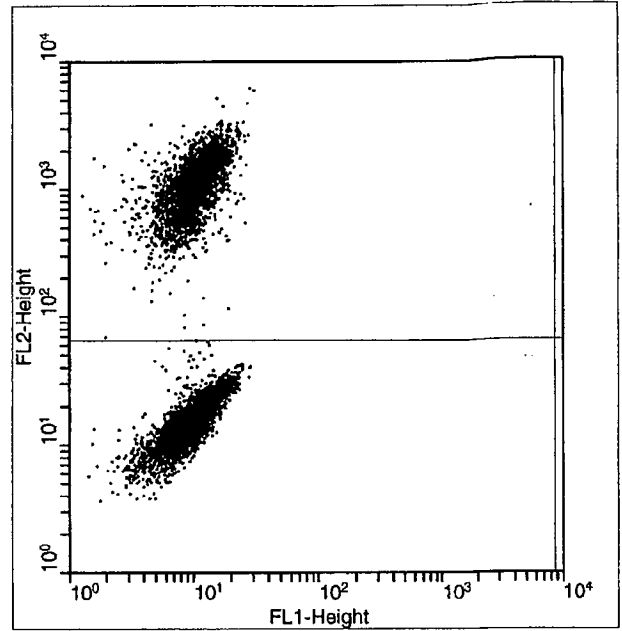
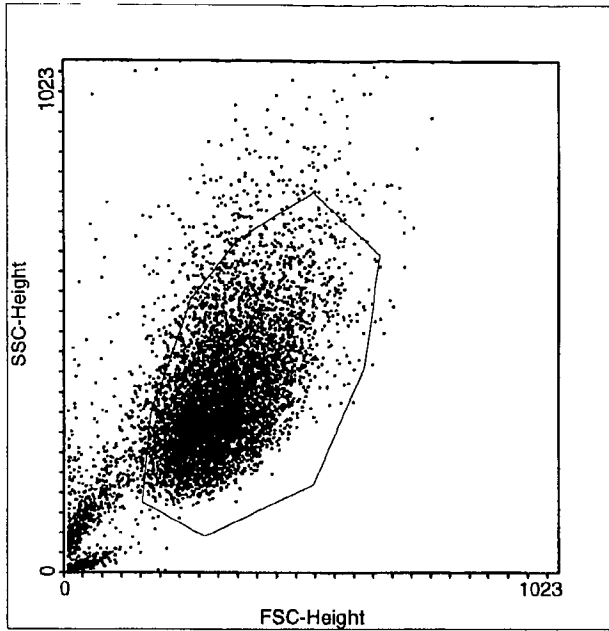
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	10.1	1039.3	3366	33.66	39.74
2 UR	0.0	0.0	0	0.00	0.00
3 LL	9.5	14.7	5103	51.03	60.26
4 LR	0.0	0.0	0	0.00	0.00

$39.74 \times 2 = 79.48 = 1.32$

$79.48 + 60.26 = 139.74$

$139.74 - 100\%$   
 $79.48 - x$

$x = \frac{79.48 \times 100}{139.74} = \frac{56.88}{43.12}$



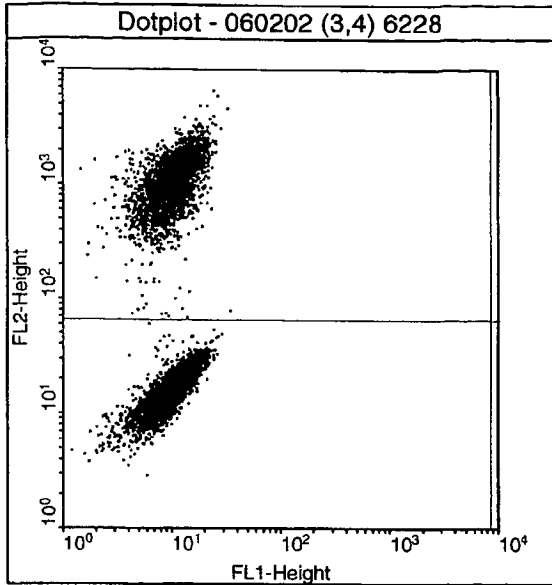
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:50:06 2002  
 Gates: R1  
 Project: Experiment:  
 File: 060002 Sample: 0Gy/0Gy(DiI)+0Gy/10Gy  
 Date: 6-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8294 82.94%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,464

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	10.1	1032.0	3348	33.48	40.37
2 UR	0.0	0.0	0	0.00	0.00
3 LL	9.4	14.7	4946	49.46	59.63
4 LR	0.0	0.0	0	0.00	0.00

$40.37 \times 2 = 80.74 = 1.35$

$80.74 + 59.63 = 140.37$

$140.37 - 100\%$   
 $80.74 - x$   
 $x = \frac{80.74 \times 100}{140.37} = \begin{array}{|l} 57.52 \\ \hline 42.48 \end{array}$



Multiple Document Interface for Flow Cytometry

WinMDI Version 2.8 - Windows 3.95/DOS 7.10

Fri Jun 07 10:51:59 2002

Gates: R1

Project: Experiment:

File: 060202 Sample: 0Gy/0Gy (DiI) + 0Gy/20Gy

Date: 6-Jun-2 Parameters: 7

Total Events 10000 Gated Events 8295 82.95%

System: Log Parameter Means: Geometric

Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 1008,464

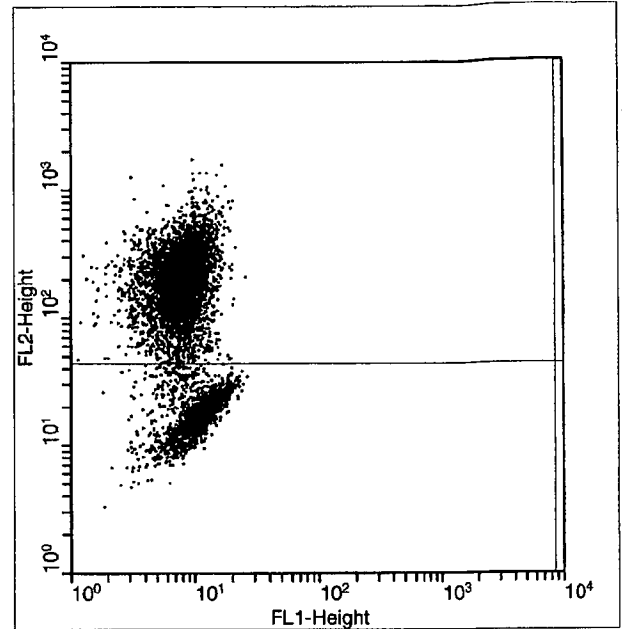
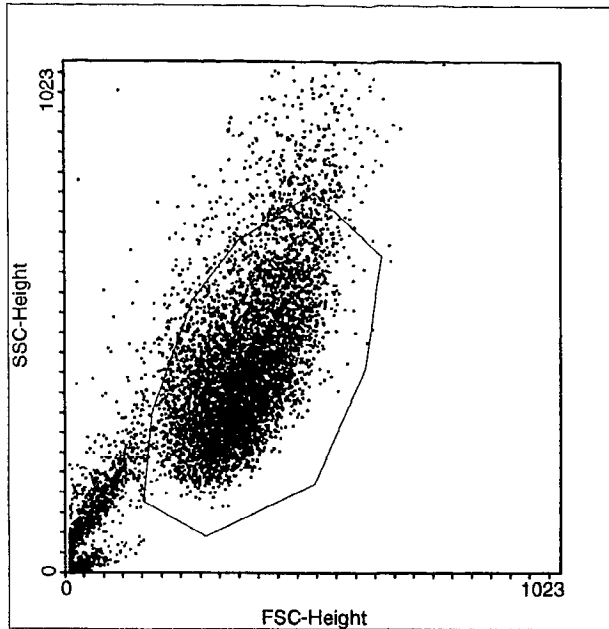
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	10.2	1015.2	3498	34.98	42.17
2 UR	0.0	0.0	0	0.00	0.00
3 LL	9.7	15.2	4797	47.97	57.83
4 LR	0.0	0.0	0	0.00	0.00

$42.17 \times 2 = 84.34$   $\approx 1.46$

$84.34 + 57.83 = 142.17$

$142.17 - 100$   
 $84.34 - x$

$x = \frac{84.34 \times 100}{142.17} = \frac{59.32}{40.68}$

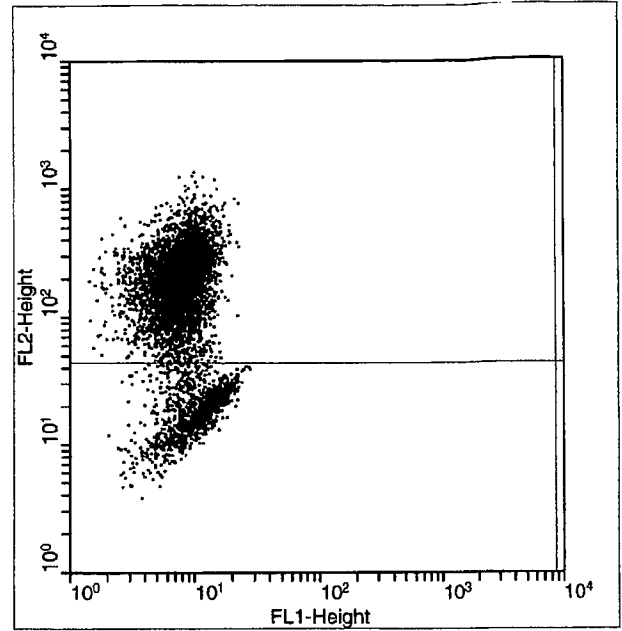
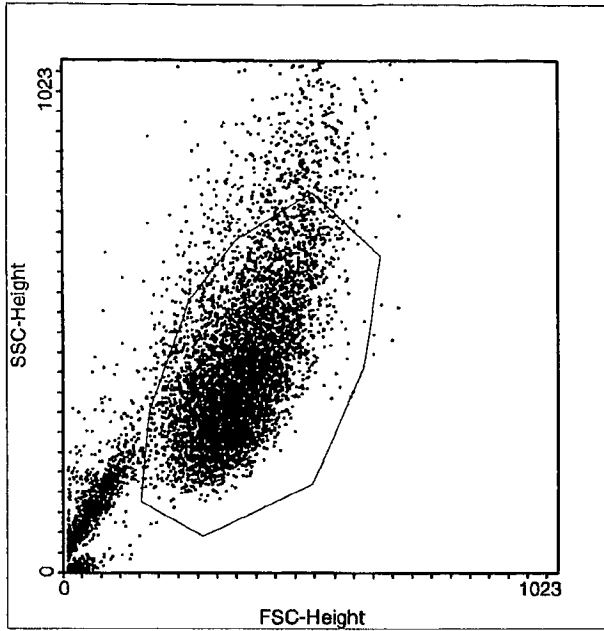


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:55:23 2002

Gates: R1  
 Project: Experiment:  
 File: 060002 Sample: (0Gy (DiI) +5Gy)  
 Date: 6-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 7649 76.49%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,420

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.8	194.2	5665	56.65	74.06
2 UR	0.0	0.0	0	0.00	0.00
3 LL	10.1	17.5	1984	19.84	25.94
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:56:04 2002

Gates: R1

Project: Experiment:  
 File: 060002 Sample: (0Gy (DiI) +10Gy)

Date: 6-Jun-2 Parameters: 7

Total Events 10000 Gated Events 7532 75.32%

System: Log Parameter Means: Geometric

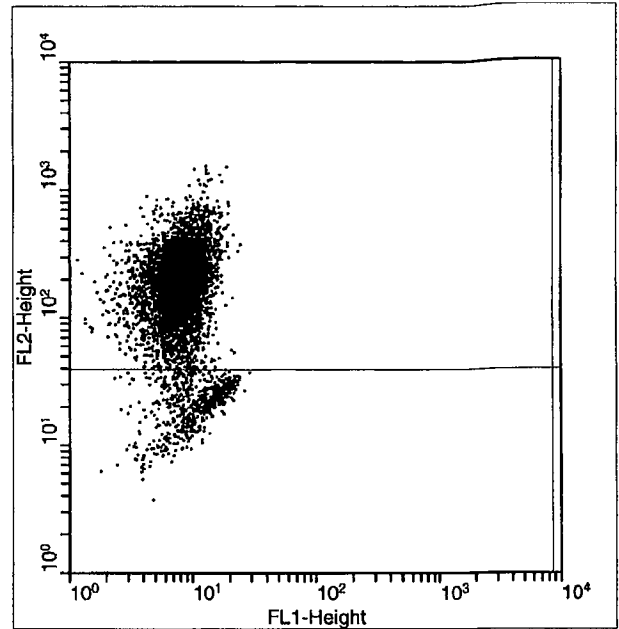
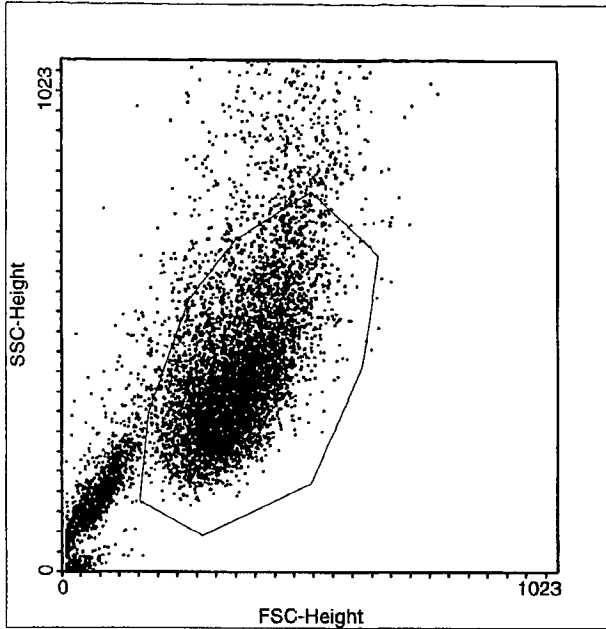
Quad Stats

FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 1008,420

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.6	193.5	5947	59.47	78.96
2 UR	0.0	0.0	0	0.00	0.00
3 LL	10.0	18.3	1585	15.85	21.04
4 LR	0.0	0.0	0	0.00	0.00





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 07 10:57:11 2002

Gates: R1  
 Project: Experiment:  
 File: 060602 Sample: (0Gy(DiI)+20Gy)  
 Date: 6-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 7135 71.35%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,408

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.8	189.4	6317	63.17	88.54
2 UR	0.0	0.0	0	0.00	0.00
3 LL	10.2	20.9	818	8.18	11.46
4 LR	0.0	0.0	0	0.00	0.00

JUNE 7, 2002

CALCULATIONS (MICROSCOPY)

① AMOUNTS OF UNIRRADIATED CELLS IN THE CO-CULTURES WITH IRRADIATED CELLS

$$\frac{0\text{Gy}^*/10\text{Gy}}{668 \times 10^3 \times 49.4\%} = \frac{330 \times 10^3 \text{ cells}}{100\%}$$

$$\frac{0\text{Gy}^*/0.5\text{Gy}}{563 \times 10^3 \times 55.2\%} = \frac{311 \times 10^3 \text{ cells}}{100\%}$$

$$\frac{0\text{Gy}^*/1\text{Gy}}{671 \times 10^3 \times 59.2\%} = \frac{397 \times 10^3 \text{ cells}}{100\%}$$

$$\frac{0\text{Gy}^*/5\text{Gy}}{534 \times 10^3 \times 70.8\%} = \frac{378 \times 10^3 \text{ cells}}{100\%}$$

$$\frac{0\text{Gy}^*/10\text{Gy}}{411 \times 10^3 \times 75.8\%} = \frac{312 \times 10^3 \text{ cells}}{100\%}$$

$$\frac{0\text{Gy}^*/20\text{Gy}}{409 \times 10^3 \times 81.8\%} = \frac{335 \times 10^3 \text{ cells}}{100\%}$$

② AMOUNTS OF IRRADIATED CELLS IN THE CO-CULTURES WITH IRRADIATED CELLS

$$\frac{0\text{Gy}^*/0\text{Gy}}{668 \times 10^3 \times 50.6\%} = \frac{338 \times 10^3 \text{ cells}}{100\%}$$

$$\frac{0\text{Gy}^*/0.5\text{Gy}}{563 \times 10^3 \times 44.8\%} = \frac{252 \times 10^3 \text{ cells}}{100\%}$$

$$\frac{0\text{Gy}^*/1\text{Gy}}{671 \times 10^3 \times 40.8\%} = \frac{277 \times 10^3 \text{ cells}}{100\%}$$

$$\frac{0\text{Gy}^*/5\text{Gy}}{534 \times 10^3 \times 29.2\%} = \frac{156 \times 10^3 \text{ cells}}{100\%}$$

$$\frac{0\text{Gy}^*/10\text{Gy}}{411 \times 10^3 \times 24.2\%} = \frac{99 \times 10^3 \text{ cells}}{100\%}$$

$$0\text{Gy}^* / 20\text{Gy}$$

$$\frac{409 \times 10^3 \times 18.2\%}{100\%} = 74 \times 10^3 \text{ cells}$$

③ AMOUNTS OF UNIRRADIATED AND IRRADIATED CELLS CULTURED SEPARATELY

$$0\text{Gy}^* + 5\text{Gy}$$

$$\frac{399 \times 10^3 \times 74.1\%}{100\%} = 296 \times 10^3 \text{ cells}$$

$$0\text{Gy}^* + 5\text{Gy}$$

$$\frac{399 \times 10^3 \times 25.9\%}{100\%} = 103 \times 10^3 \text{ cells}$$

$$0\text{Gy}^* + 10\text{Gy}$$

$$\frac{392 \times 10^3 \times 79.0\%}{100\%} = 310 \times 10^3 \text{ cells}$$

$$0\text{Gy}^* + 10\text{Gy}$$

$$\frac{392 \times 10^3 \times 21.0\%}{100\%} = 82 \times 10^3 \text{ cells}$$

$$0\text{Gy}^* + 20\text{Gy}$$

$$\frac{313 \times 10^3 \times 88.5\%}{100\%} = 277 \times 10^3 \text{ cells}$$

$$0\text{Gy}^* + 20\text{Gy}$$

$$\frac{313 \times 10^3 \times 11.5\%}{100\%} = 36 \times 10^3 \text{ cells}$$

CALCULATIONS (FCM)

$$\textcircled{1} \frac{0.6\text{Gy}(X_1) + 0.6\text{Gy}(Y_1)}{0.6\text{Gy}(X_2) + 0.56\text{Gy}(Y_2)} = \frac{49.83}{50.17} = 0.99$$

$$X_1(49.36) + Y_1(50.64) = 100 \text{ events} \text{ --- CONTROL}$$

$$\frac{100}{0.6\text{Gy}(X_2) + 0.56\text{Gy}(Y_2)} = 0.99$$

$$0.6\text{Gy}(X_2) + 0.56\text{Gy}(Y_2) = 101$$

$$\text{CONVERTED } X_2 = \frac{101 \times 55.23\%}{100\%} = 55.8 \rightarrow 0.6\text{Gy}$$

$$\text{CONVERTED } Y_2 = \frac{101 \times 44.77\%}{100\%} = 45.2 \rightarrow 0.56\text{Gy}$$

$$\textcircled{2} \frac{51.98}{48.02} = 1.08 \quad \frac{100}{1.08} = 92.6$$

$$\text{CONVERTED } X_2 = \frac{92.6 \times 59.18\%}{100\%} = 54.8 \rightarrow 0.6\text{Gy}$$

$$\text{CONVERTED } Y_2 = \frac{92.6 \times 40.82\%}{100\%} = 37.8 \rightarrow 1.6\text{Gy}$$

$$\textcircled{3} \frac{56.88}{43.12} = 1.32 \quad \frac{100}{1.32} = 75.8$$

$$\text{CONVERTED } X_2 = \frac{75.8 \times 70.80\%}{100\%} = 53.7 \rightarrow 0.6\text{Gy}$$

$$\text{CONVERTED } Y_2 = \frac{75.8 \times 29.20\%}{100\%} = 22.1 \rightarrow 5.6\text{Gy}$$

$$\textcircled{4} \frac{57.52}{42.48} = 1.35 \quad \frac{100}{1.35} = 74.1$$

$$\text{CONVERTED } X_2 = \frac{74.1 \times 75.83\%}{100\%} = 56.2 \rightarrow 0.6\text{Gy}$$

$$\text{CONVERTED } Y_2 = \frac{74.1 \times 24.17\%}{100\%} = 17.9 \rightarrow 10\text{Gy}$$

$$\textcircled{5} \quad \frac{59.32}{40.68} = 1.46 \quad \frac{100}{1.46} = 68.5$$

$$\text{CONVERTED } X_2 = \frac{68.5 \times 81.83\%}{100\%} = 56.1 \longrightarrow 067$$

$$\text{CONVERTED } Y_2 = \frac{68.5 \times 18.17\%}{100\%} = 12.4 \longrightarrow 2067$$

#3

JUNE 9, 2002

BYSTANDER EFFECTS (γ-IRRADIATION)

(WB/22)

100% CONFLUENCY

- 0 Gy
- 0 Gy
- 0 Gy
- 0.5 Gy
- 1.0 Gy
- 5.0 Gy
- 10.0 Gy
- 20.0 Gy

FOR DiI staining (40min)

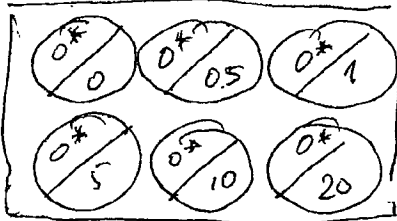
IRRADIATION TIME: 9:30

ONE SOURCE: 345.59 Rad/min

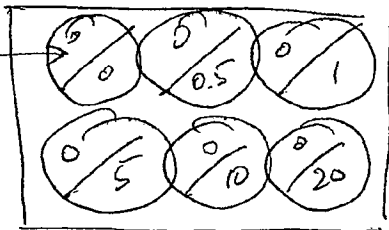
100 μL / 100 μL SCHEME

PLATING TIME: 11:00

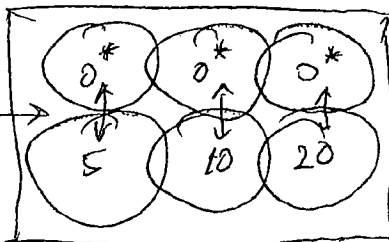
Note: 0\* - 0 Gy - DiI stained



To be stained prior to FCM



To be combined prior to FCM



$0.6\text{g} \rightarrow 1.5\text{ml}$ 
 $\left. \begin{matrix} 485 \\ 520 \end{matrix} \right\} 1005 \times 10^3 \text{ cells/ml} \times \text{dilution factor}$   
 $2010 \times 10^3 \text{ cells/ml}$

$\phi 5 \text{ dish or } 1.5\text{ml} \leftarrow 3.02 \times 10^6 \text{ cells}$

PLATED:  $100\mu\text{L} \leftarrow \frac{201}{2} \times 10^3 \text{ cells}$   
 OR:  $100\mu\text{L} + 100\mu\text{L} \rightarrow \frac{402}{2} \times 10^3 \text{ cells}$

JUNE 10, 2002

Hemocytometer Counting

$0.6\text{g}^*/0.6\text{g}$ 
 $\left. \begin{matrix} 198 \\ 173 \end{matrix} \right\} 371$ 
 $\left. \begin{matrix} 200 \\ 204 \end{matrix} \right\} 404$ 
 $\left. \begin{matrix} 371 \\ 404 \end{matrix} \right\} \frac{775}{2} = 388 \times 10^3 \text{ cells/ml}$   
 $+0.5\text{ml} \rightarrow 582 \times 10^3 \text{ cells}$

$0.6\text{g}^*/0.5\text{Gy}$ 
 $\left. \begin{matrix} 197 \\ 203 \end{matrix} \right\} 400$ 
 $\left. \begin{matrix} 201 \\ 189 \end{matrix} \right\} 390$ 
 $\left. \begin{matrix} 400 \\ 390 \end{matrix} \right\} \frac{790}{2} = 395 \times 10^3 \text{ cells/ml}$   
 $+0.5\text{ml} \rightarrow 593 \times 10^3 \text{ cells}$

$0.6\text{g}^*/1.6\text{g}$ 
 $\left. \begin{matrix} 194 \\ 211 \end{matrix} \right\} 405$ 
 $\left. \begin{matrix} 208 \\ 195 \end{matrix} \right\} 403$ 
 $\left. \begin{matrix} 405 \\ 403 \end{matrix} \right\} \frac{808}{2} = 404 \times 10^3 \text{ cells/ml}$   
 $+0.5\text{ml} \rightarrow 606 \times 10^3 \text{ cells}$

$0.6\text{g}^*/5.6\text{g}$ 
 $\left. \begin{matrix} 166 \\ 139 \end{matrix} \right\} 305$ 
 $\left. \begin{matrix} 174 \\ 159 \end{matrix} \right\} 333$ 
 $\left. \begin{matrix} 305 \\ 333 \end{matrix} \right\} \frac{638}{2} = 319 \times 10^3 \text{ cells/ml}$   
 $+0.5\text{ml} \rightarrow 479 \times 10^3 \text{ cells}$

$0.6\text{g}^*/10.6\text{g}$ 
 $\left. \begin{matrix} 182 \\ 172 \end{matrix} \right\} 354$ 
 $\left. \begin{matrix} 135 \\ 186 \end{matrix} \right\} 321$ 
 $\left. \begin{matrix} 354 \\ 321 \end{matrix} \right\} \frac{675}{2} = 338 \times 10^3 \text{ cells/ml}$   
 $+0.5\text{ml} \rightarrow 507 \times 10^3 \text{ cells}$

$0.6\text{g}^*/20$ 
 $\left. \begin{matrix} 161 \\ 171 \end{matrix} \right\} 332$ 
 $\left. \begin{matrix} 140 \\ 167 \end{matrix} \right\} 307$ 
 $\left. \begin{matrix} 332 \\ 307 \end{matrix} \right\} \frac{639}{2} = 320 \times 10^3 \text{ cells/ml}$   
 $+0.5\text{ml} \rightarrow 480 \times 10^3 \text{ cells}$



$0.6\text{Gy}^* + 5.6\text{Gy}$ :
 
$$\left. \begin{array}{l} 175 \\ 145 \\ 156 \\ 157 \end{array} \right\} \begin{array}{l} 320 \\ 313 \end{array} \left. \vphantom{\begin{array}{l} 175 \\ 145 \\ 156 \\ 157 \end{array}} \right\} \frac{633}{2} = 317 \times 10^3 \text{ cells/mL}$$

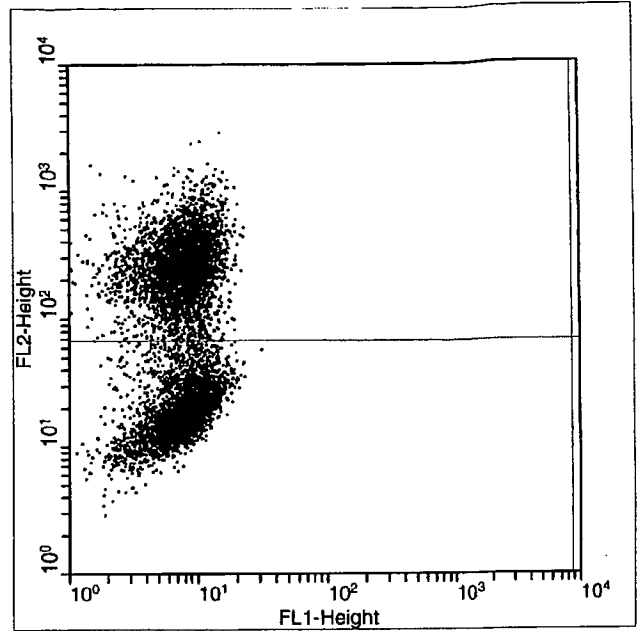
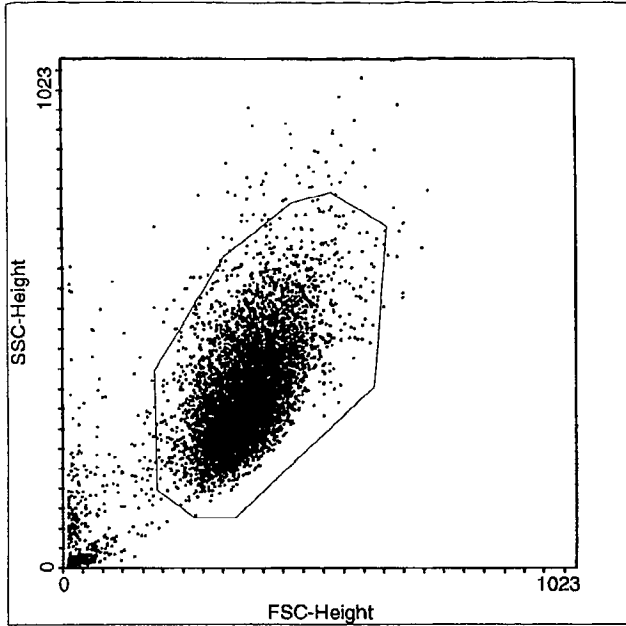
$$+ 0.5 \text{ mL} \longrightarrow 475 \times 10^3 \text{ cells}$$

$0.6\text{Gy}^* + 10.6\text{Gy}$ :
 
$$\left. \begin{array}{l} 141 \\ 132 \\ 132 \\ 132 \end{array} \right\} \begin{array}{l} 273 \\ 264 \end{array} \left. \vphantom{\begin{array}{l} 141 \\ 132 \\ 132 \\ 132 \end{array}} \right\} \frac{537}{2} = 269 \times 10^3 \text{ cells/mL}$$

$$+ 0.5 \text{ mL} \longrightarrow 403 \times 10^3 \text{ cells}$$

$0.6\text{Gy}^* + 20.6\text{Gy}$ :
 
$$\left. \begin{array}{l} 166 \\ 111 \\ 115 \\ 138 \end{array} \right\} \begin{array}{l} 277 \\ 253 \end{array} \left. \vphantom{\begin{array}{l} 166 \\ 111 \\ 115 \\ 138 \end{array}} \right\} \frac{530}{2} = 265 \times 10^3 \text{ cells/mL}$$

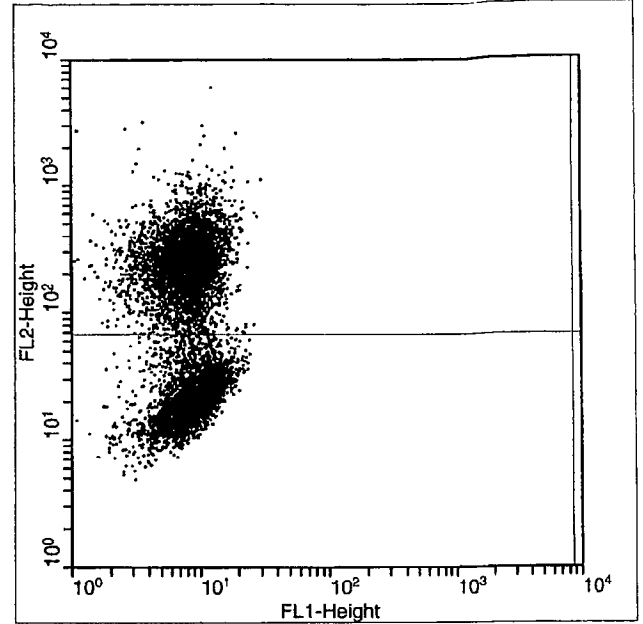
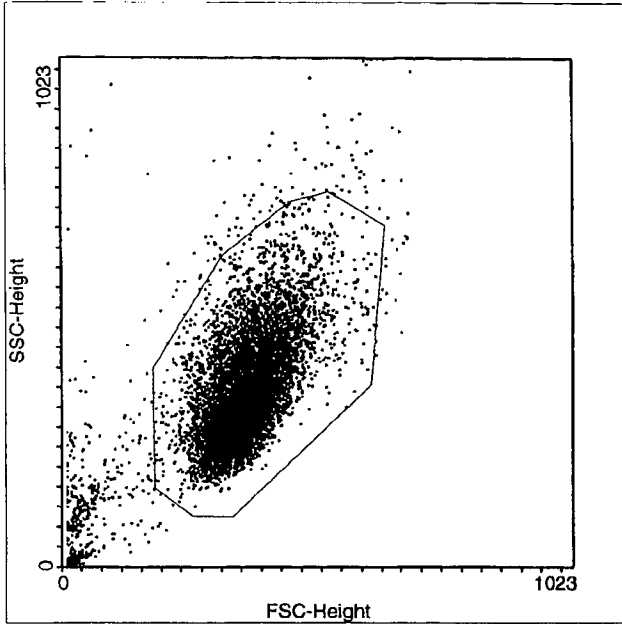
$$+ 0.5 \text{ mL} \longrightarrow 398 \times 10^3 \text{ cells}$$



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:08:18 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: OCy(DiI)/OGy  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9263 92.63%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,468

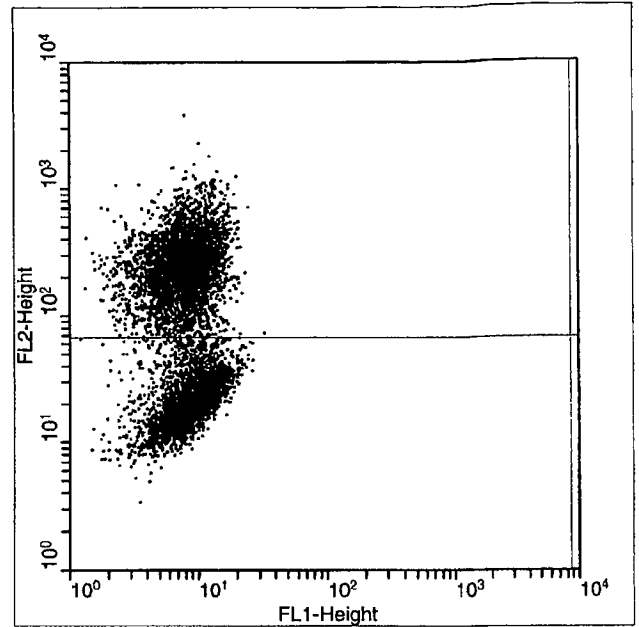
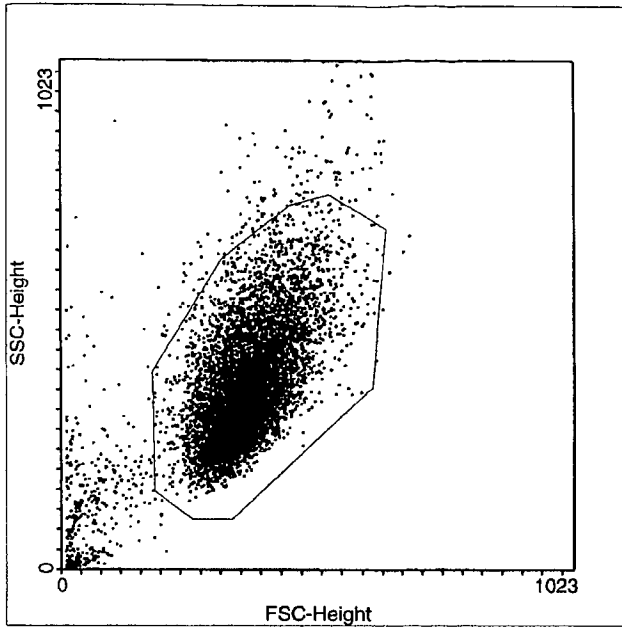
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.5	255.8	4759	47.59	51.38
2 UR	0.0	0.0	0	0.00	0.00
3 LL	7.5	18.9	4504	45.04	48.62
4 LR	0.0	0.0	0	0.00	0.00

= 1.06



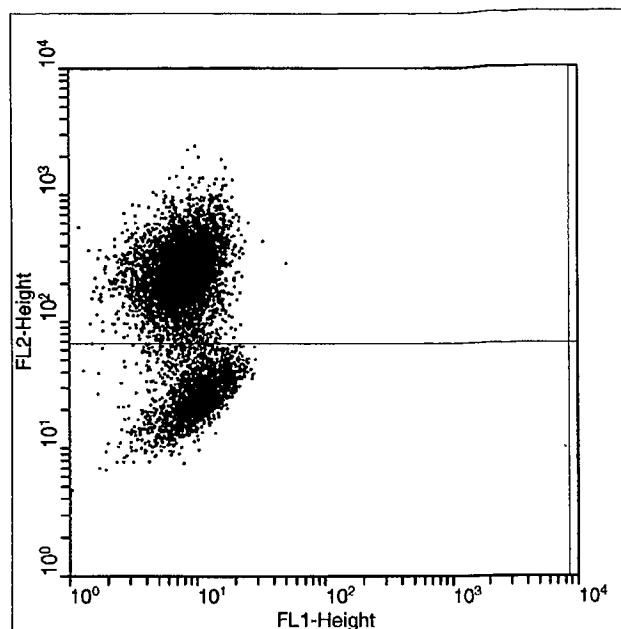
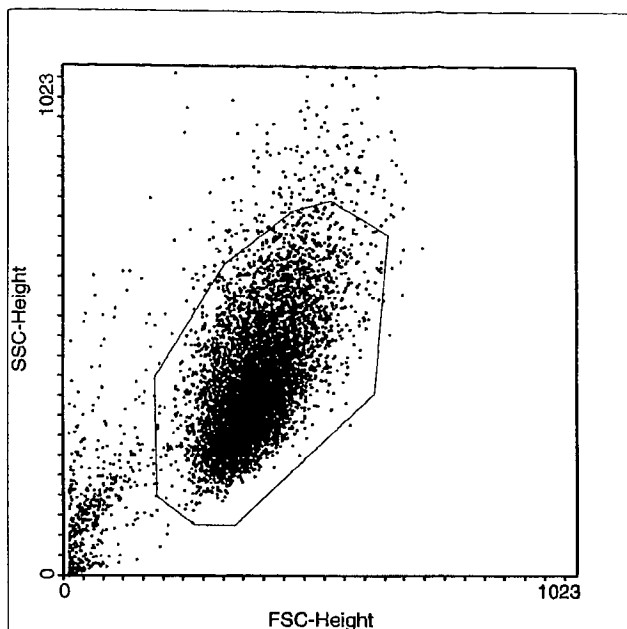
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:12:47 2002  
 Gates: R1  
 Project: Experiment: 0.5  
 File: 061002 Sample: 0Gy(DiI)/0Gy  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9240 92.40%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,468

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.7	252.1	5216	52.16	56.45
2 UR	0.0	0.0	0	0.00	0.00
3 LL	8.5	21.3	4024	40.24	43.55
4 LR	0.0	0.0	0	0.00	0.00



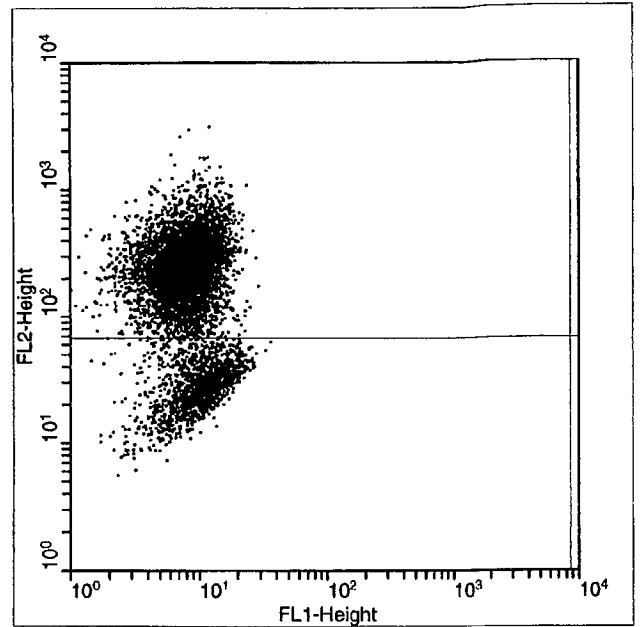
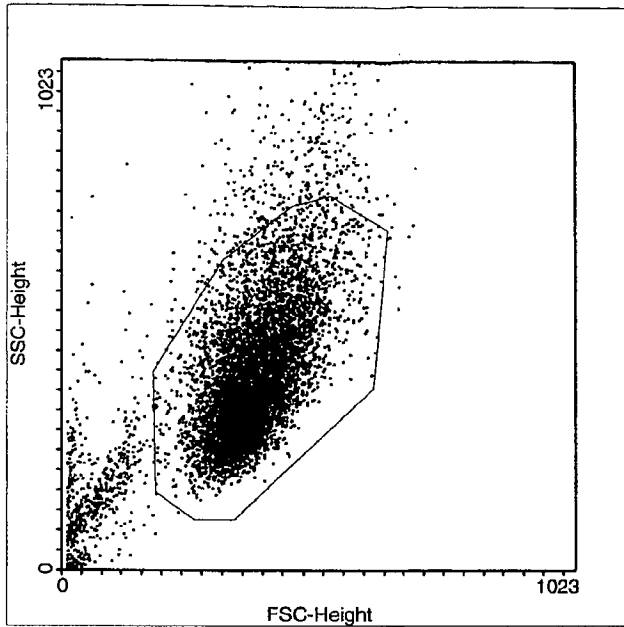
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:13:53 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: 0Gy(DiI)/1Gy  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9246 92.46%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,468

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.7	246.7	5471	54.71	<u>59.17</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	8.6	21.8	3775	37.75	<u>40.83</u>
4 LR	0.0	0.0	0	0.00	0.00



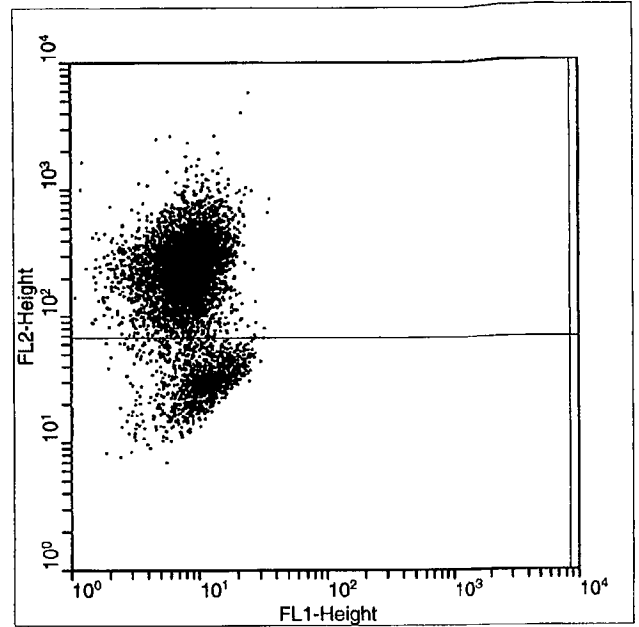
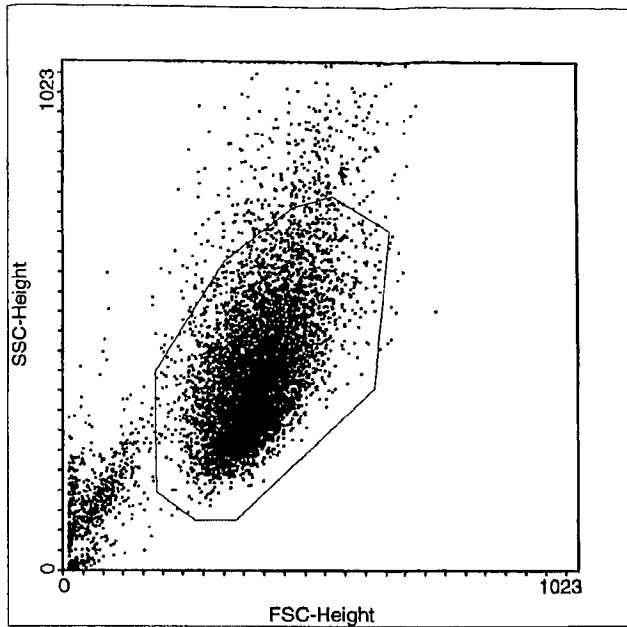
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:15:23 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: 0Gy(DiI)/5Gy  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9002 90.02%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,468

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.9	248.0	6445	64.45	<u>71.60</u>
2 UR	0.0	0.0	0	0.00	<u>0.00</u>
3 LL	9.8	26.7	2557	25.57	<u>28.40</u>
4 LR	0.0	0.0	0	0.00	<u>0.00</u>



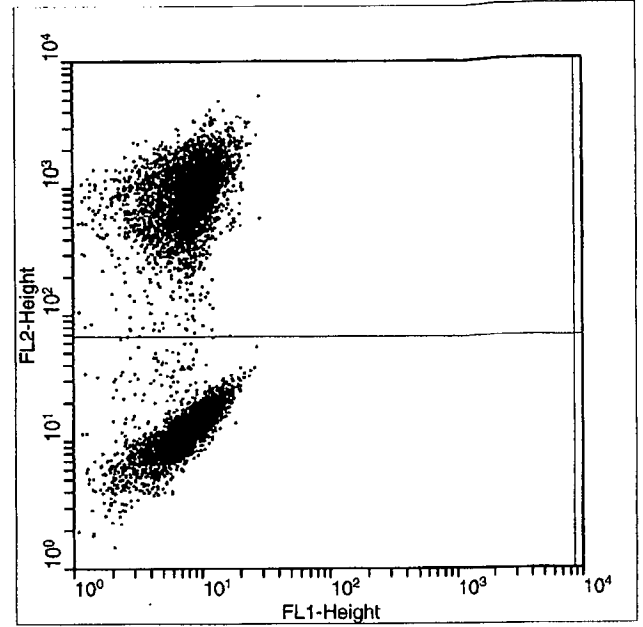
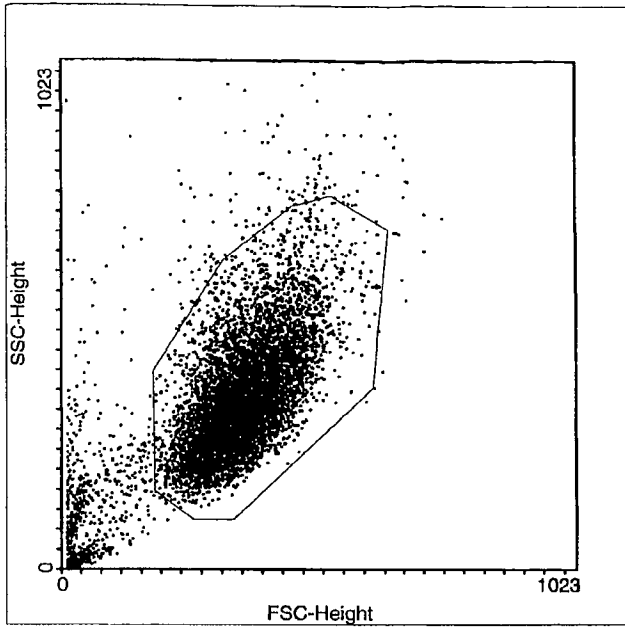
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:16:08 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: 0Gy(DiI)/10Gy  
 Date: 10-Jun-2 Parameters:  
 Total Events 10000 Gated Events 8702 87.02%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,468  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.7	251.1	6705	67.05	<u>77.05</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	10.0	28.0	1997	19.97	<u>22.95</u>
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:16:54 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: (0Gy(DiI)/20Gy)  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8509 85.09%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,468  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.9	244.7	6965	69.65	81.85
2 UR	0.0	0.0	0	0.00	0.00
3 LL	10.4	31.4	1544	15.44	18.15
4 LR	0.0	0.0	0	0.00	0.00

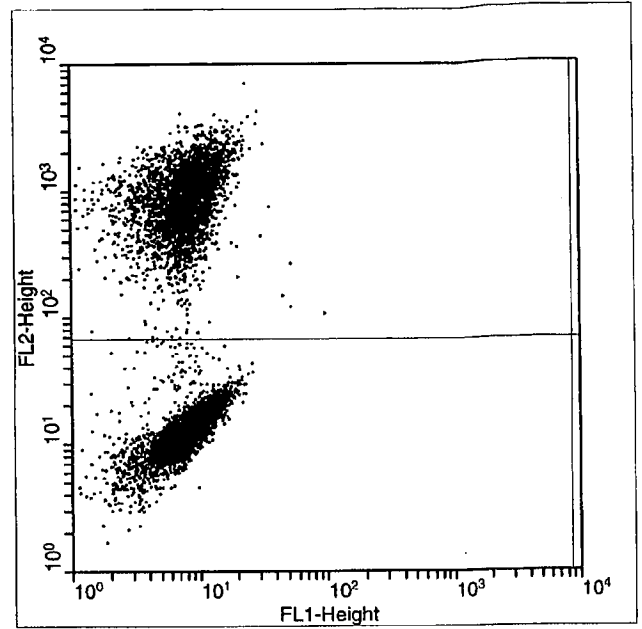
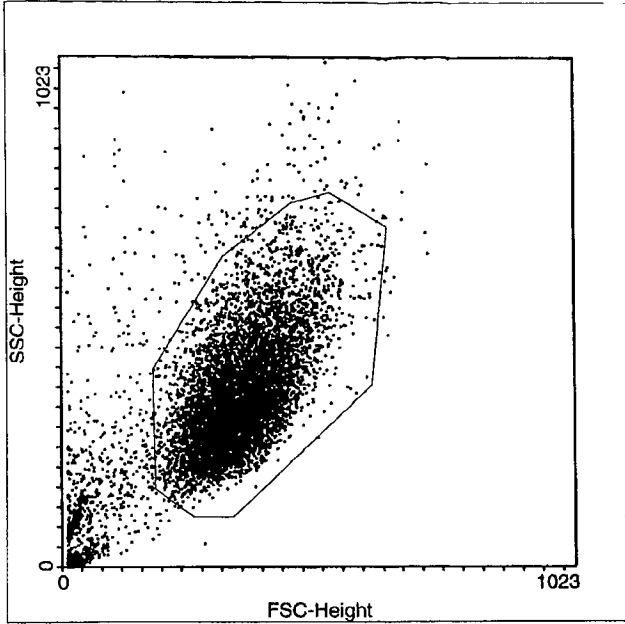


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:18:24 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: 0Gy/0Gy(DiI)+0Gy/0.5Gy  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8834 88.34%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,468

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.8	865.5	4600	46.00	52.07
2 UR	0.0	0.0	0	0.00	0.00
3 LL	7.2	11.9	4234	42.34	47.93
4 LR	0.0	0.0	0	0.00	0.00

1.09

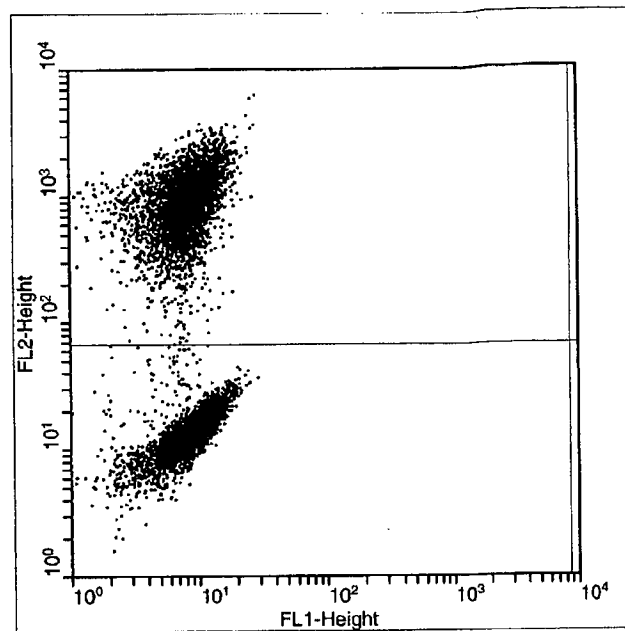
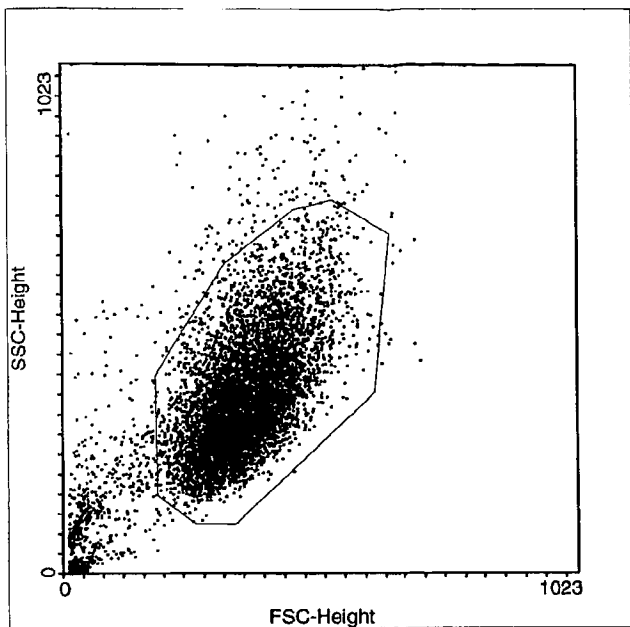




Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:19:16 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: 0Gy/0Gy(DiI)+0Gy/1Gy  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8759 87.59%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,468

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.9	860.1	4330	43.30	49.43
2 UR	0.0	0.0	0	0.00	0.00
3 LL	7.5	12.3	4429	44.29	50.57
4 LR	0.0	0.0	0	0.00	0.00

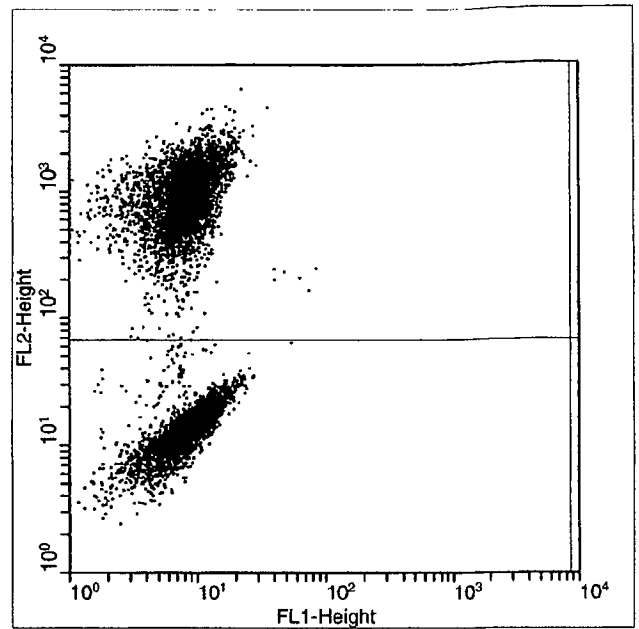
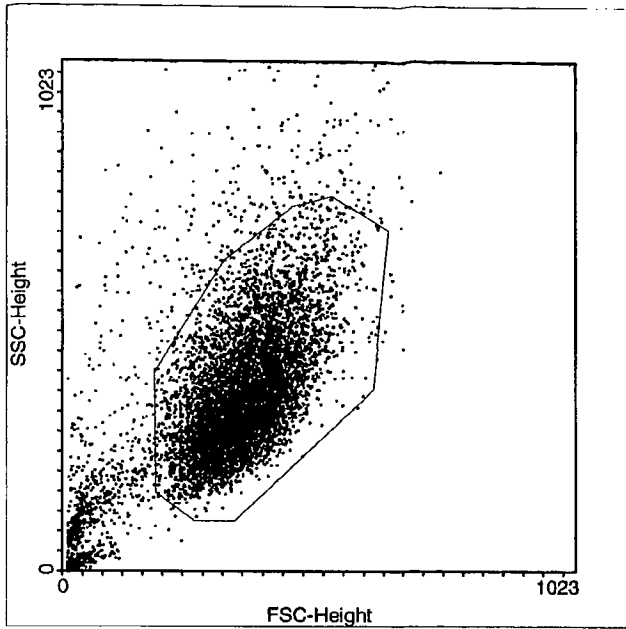
0.98



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:26:15 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: 0Gy/0Gy (DIT)+0Gy/5Gy  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8843 88.43%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,468

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	8.1	854.5	4820	48.20	54.51
2 UR	0.0	0.0	0	0.00	0.00
3 LL	7.9	13.0	4023	40.23	45.49
4 LR	0.0	0.0	0	0.00	0.00

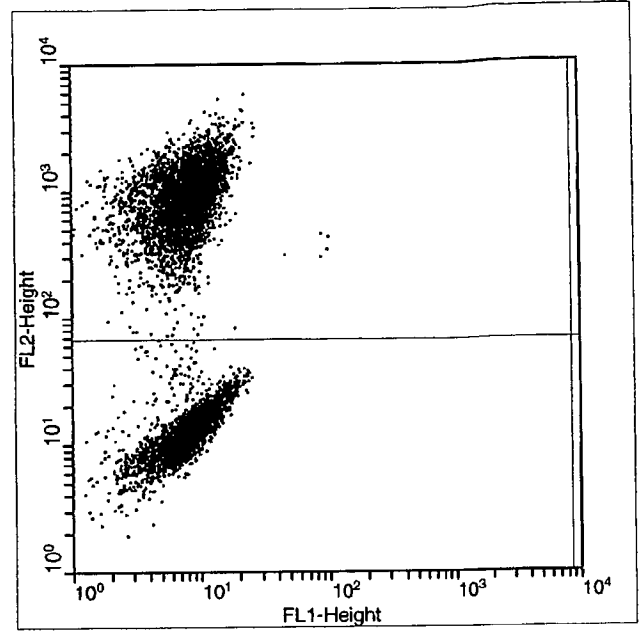
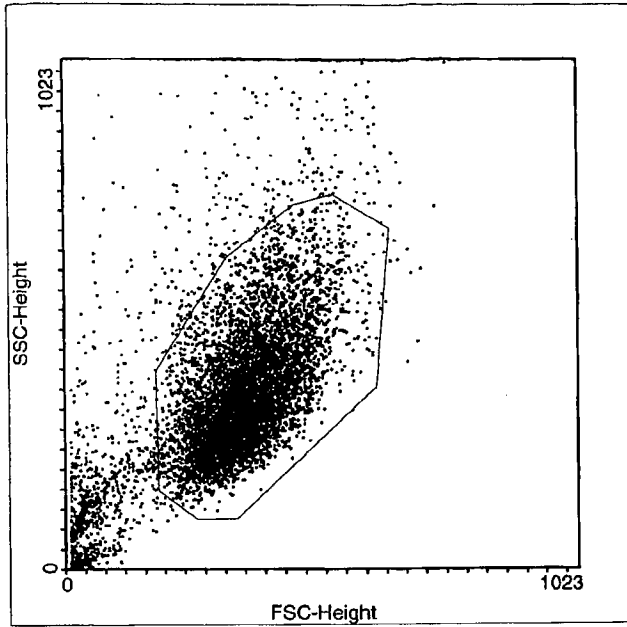
(1.2)



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:27:00 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: 0Gy/0Gy(DiI)+0Gy/10Gy  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8622 86.22%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,468

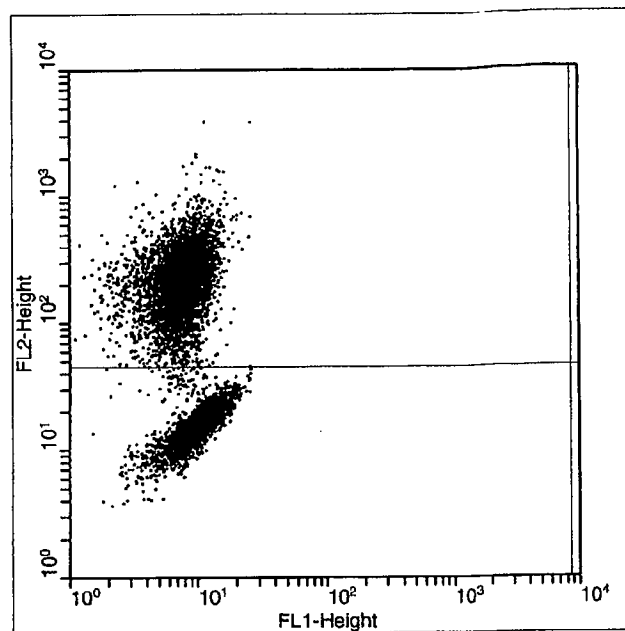
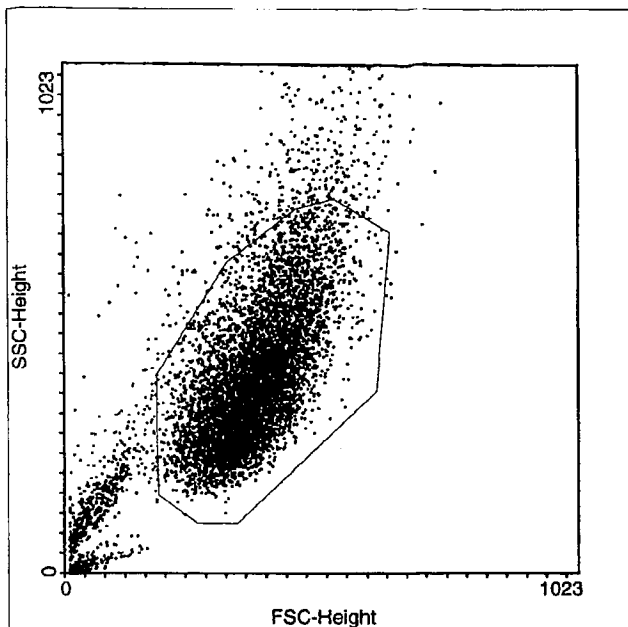
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.9	876.3	4744	47.44	55.02
2 UR	0.0	0.0	0	0.00	0.00
3 LL	7.8	12.8	3878	38.78	44.98
4 LR	0.0	0.0	0	0.00	0.00

(1.22)



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:27:39 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: 0Gy/0Gy(DiI)+0Gy/20Gy  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8496 84.96%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,468

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.9	867.6	4940	49.40	58.15
2 UR	0.0	0.0	0	0.00	0.00
3 LL	7.9	13.0	3556	35.56	41.85
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:28:33 2002

Gates: R1

Project: Experiment:

File: 061002 Sample: 0Gy (DiI)+5Gy

Date: 10-Jun-2 Parameters: 7

Total Events 10000 Gated Events 8505 85.05%

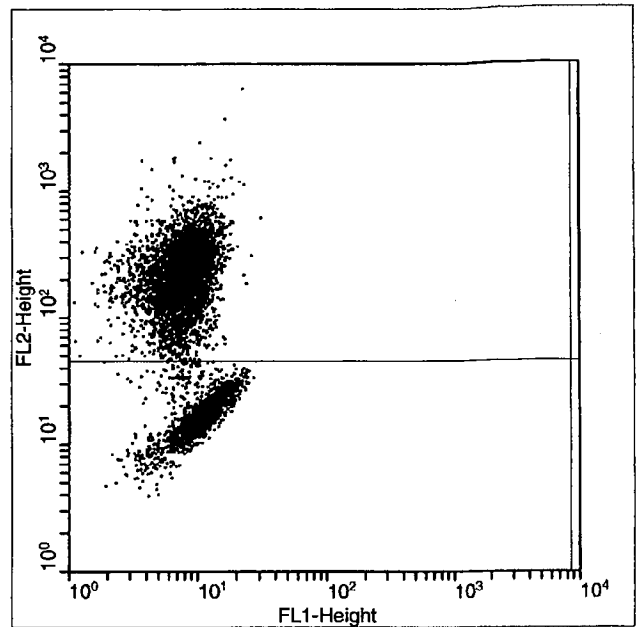
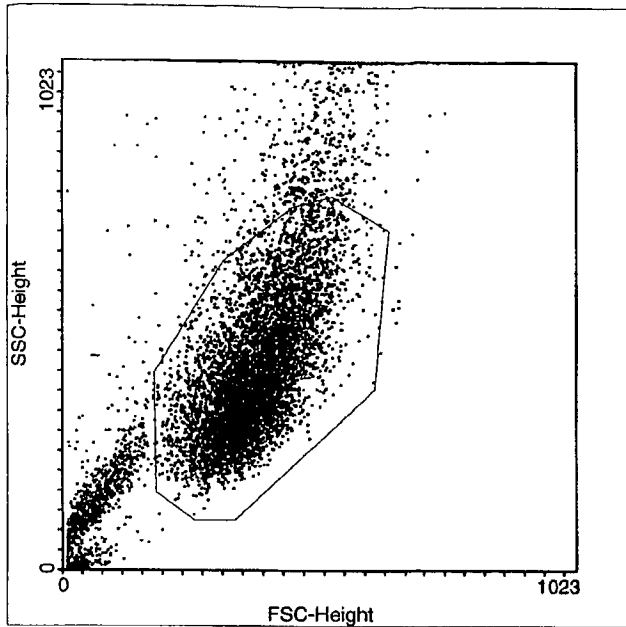
System: Log Parameter Means: Geometric

Quad Stats

FL1-Height (Log) vs FL2-Height (Log)

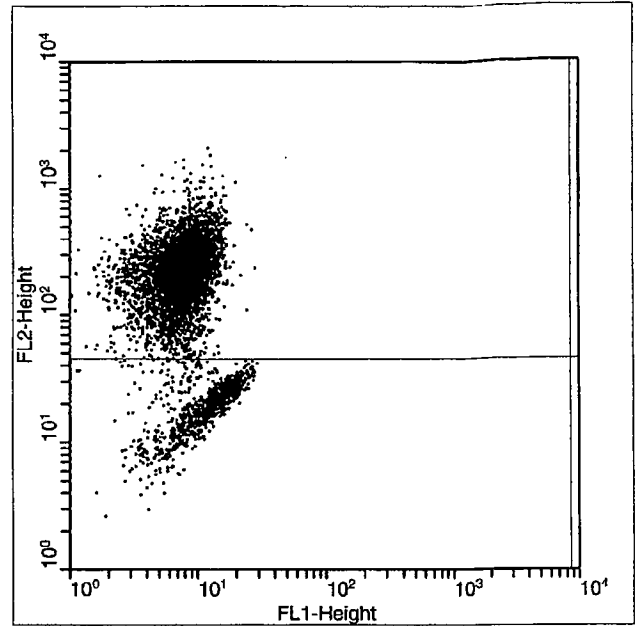
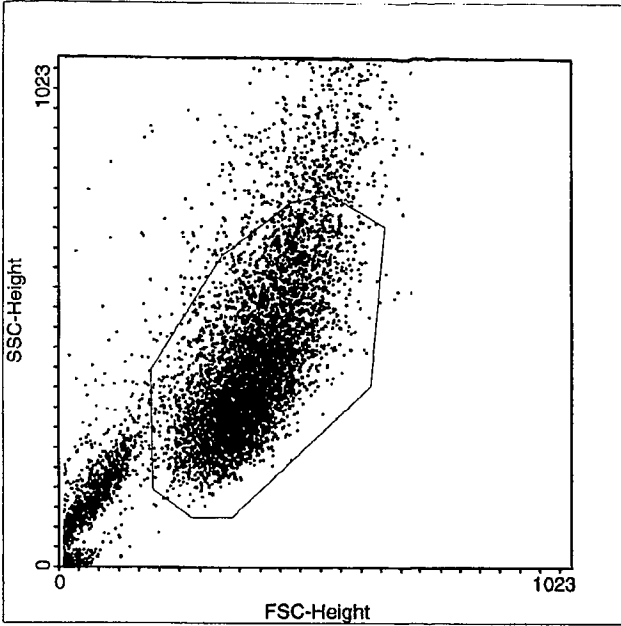
Quadrant x,y: 1008,424

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.5	213.8	5938	59.38	69.82
2 UR	0.0	0.0	0	0.00	0.00
3 LL	9.8	15.7	2567	25.67	30.18
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:29:00 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: 0Gy(DiI)+10Gy  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 7859 78.59%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,424  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.7	216.0	6059	60.59	<u>77.10</u>
2 UR	0.0	0.0	0	0.00	<u>0.00</u>
3 LL	10.5	17.3	1800	18.00	<u>22.90</u>
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Tue Jun 11 10:29:39 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061002 Sample: 0Gy(DiI)+20Gy  
 Date: 10-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 7526 75.26%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,424

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	7.5	217.2	6338	63.38	84.21
2 UR	0.0	0.0	0	0.00	0.00
3 LL	11.0	19.0	1188	11.88	15.79
4 LR	0.0	0.0	0	0.00	0.00

JUNE 12, 2002

CALCULATIONS (MICROSCOPY)

① AMOUNTS OF UNIRRADIATED CELLS IN THE CO-CULTURE WITH IRRADIATED CELLS

$$\frac{0\text{Gy}^* / 0\text{Gy}}{100\%} : \frac{582 \times 10^3 \times 51.4\%}{100\%} = 299 \times 10^3 \text{ cells}$$

$$\frac{0\text{Gy}^* / 0.5\text{Gy}}{100\%} : \frac{593 \times 10^3 \times 56.5\%}{100\%} = 335 \times 10^3 \text{ cells}$$

$$\frac{0\text{Gy}^* / 1\text{Gy}}{100\%} : \frac{606 \times 10^3 \times 59.2\%}{100\%} = 359 \times 10^3 \text{ cells}$$

$$\frac{0\text{Gy}^* / 5\text{Gy}}{100\%} : \frac{479 \times 10^3 \times 71.6\%}{100\%} = 343 \times 10^3 \text{ cells}$$

$$\frac{0\text{Gy}^* / 10\text{Gy}}{100\%} : \frac{507 \times 10^3 \times 77.1\%}{100\%} = 391 \times 10^3 \text{ cells}$$

$$\frac{0\text{Gy}^* / 20\text{Gy}}{100\%} : \frac{480 \times 10^3 \times 81.9\%}{100\%} = 393 \times 10^3 \text{ cells}$$

② AMOUNTS OF IRRADIATED CELLS IN THE CO-CULTURE WITH UNIRRADIATED CELLS

$$\frac{0\text{Gy}^* / 0\text{Gy}}{100\%} : \frac{582 \times 10^3 \times 48.6\%}{100\%} = 283 \times 10^3 \text{ cells}$$

$$\frac{0\text{Gy}^* / 0.5\text{Gy}}{100\%} : \frac{593 \times 10^3 \times 43.6\%}{100\%} = 259 \times 10^3 \text{ cells}$$

$$\frac{0\text{Gy}^* / 1\text{Gy}}{100\%} : \frac{606 \times 10^3 \times 40.8\%}{100\%} = 247 \times 10^3 \text{ cells}$$

$$\frac{0\text{Gy}^* / 5\text{Gy}}{100\%} : \frac{479 \times 10^3 \times 28.4\%}{100\%} = 136 \times 10^3 \text{ cells}$$

$$\frac{0\text{Gy}^* / 10\text{Gy}}{100\%} : \frac{507 \times 10^3 \times 23.0\%}{100\%} = 117 \times 10^3 \text{ cells}$$



$$0 \text{ Gy}^* / (20 \text{ Gy}) : \frac{480 \times 10^3 \times 18.2\%}{100\%} = 87 \times 10^3 \text{ cells}$$

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③ AMOUNTS OF UNIRRADIATED AND IRRADIATED CELLS CULTURED SEPARATELY

$$6 \text{ Gy}^* + 5 \text{ Gy} : \frac{475 \times 10^3 \times 69.8\%}{100\%} = 332 \times 10^3 \text{ cells}$$

$$0 \text{ Gy}^* + 5 \text{ Gy} : \frac{475 \times 10^3 \times 30.2\%}{100\%} = 143 \times 10^3 \text{ cells}$$

$$0 \text{ Gy}^* + 10 \text{ Gy} : \frac{403 \times 10^3 \times 77.10\%}{100\%} = 311 \times 10^3 \text{ cells}$$

$$0 \text{ Gy}^* + 10 \text{ Gy} : \frac{403 \times 10^3 \times 22.9\%}{100\%} = 92 \times 10^3 \text{ cells}$$

$$0 \text{ Gy}^* + 20 \text{ Gy} : \frac{398 \times 10^3 \times 84.2\%}{100\%} = 335 \times 10^3 \text{ cells}$$

$$0 \text{ Gy} + 20 \text{ Gy} : \frac{398 \times 10^3 \times 15.8\%}{100\%} = 62.9 \times 10^3 \text{ cells}$$

CALCULATIONS (ECM)

$$\textcircled{1} \frac{0\text{Gy}(X_1) + 0\text{Gy}(Y_1)}{0\text{Gy}(X_2) + 0.5\text{Gy}(Y_2)} = \frac{52.07}{47.93} = 1.09$$

$$X_1(51.4) + Y_1(48.6) = 100 \text{ events} - \text{CONTROL}$$

$$\frac{100}{0\text{Gy}(X_2) + 0.5\text{Gy}(Y_2)} = 1.09$$

$$0\text{Gy}(X_2) + 0.5\text{Gy}(Y_2) = 91.7$$

events

$$\text{CONVERTED } X_2 = \frac{91.7 \times 56.5\%}{100\%} = 51.8\% \rightarrow 0\text{Gy}$$

$$\text{CONVERTED } Y_2 = \frac{91.7 \times 43.6\%}{100\%} = 40.0\% \rightarrow 0.5\text{Gy}$$

$$\textcircled{2} \frac{49.43}{50.57} = 0.98 \quad \frac{100}{0.98} = 102$$

$$\text{CONVERTED } X_2 = \frac{102 \times 59.2\%}{100\%} = 60.4 \rightarrow 0\text{Gy}$$

$$\text{CONVERTED } Y_2 = \frac{102 \times 40.8\%}{100\%} = 41.6 \rightarrow 1\text{Gy}$$

$$\textcircled{3} \frac{54.51}{45.49} = 1.2 \quad \frac{100}{1.2} = 83.3$$

$$\text{CONVERTED } X_2 = \frac{83.3 \times 71.6\%}{100\%} = 59.6 \rightarrow 0\text{Gy}$$

$$\text{CONVERTED } Y_2 = \frac{83.3 \times 28.4}{100\%} = 23.7 \rightarrow 5\text{Gy}$$

$$\textcircled{4} \quad \frac{55.02}{44.98} = 1.22 \quad \frac{100}{1.22} = 82$$

$$\text{CONVERTED } X_2 = \frac{82 \times 77.1\%}{100\%} = 63.2 \longrightarrow 067$$

$$\text{CONVERTED } Y_2 = \frac{82 \times 23\%}{100\%} = 18.9 \longrightarrow 1067$$

$$\textcircled{5} \quad \frac{58.15}{41.85} = 1.39 \quad \frac{100}{1.39} = 71.9$$

$$\text{CONVERTED } X_2 = \frac{71.9 \times 81.9\%}{100\%} = 58.9 \longrightarrow 067$$

$$\text{CONVERTED } Y_2 = \frac{71.9 \times 18.2\%}{100\%} = 13.1 \longrightarrow 2067$$

#4

BYSTANDER EFFECTS (γ-IRRADIATION)

JUNE 13, 2002

WB/23 100% Confluency

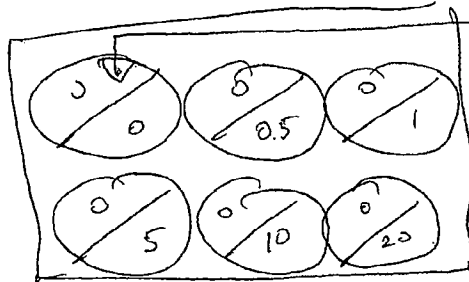
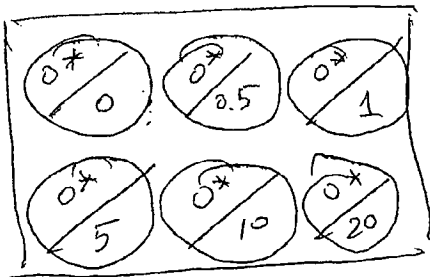
- 0 Gy
- 0 Gy
- 0 Gy - FOR DiI staining (40 min)
- 0.5
- 1
- 5
- 10
- 20

IRRADIATION TIME: 8:30

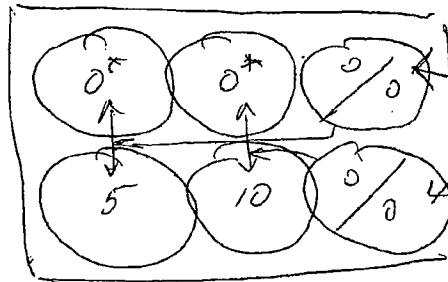
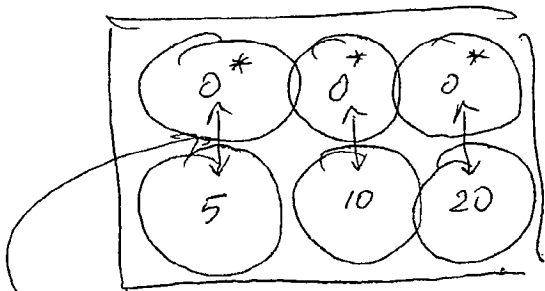
ONE SOURCE: 345.59 Rad/min

Ratio 100 μL / 100 μL

PLATING TIME: 10:00



To be stained prior to FCM



To be combined prior to FCM

Note: 0\* - 0 Gy - DiI stained

0Gy → 1.5 mL }  $\left. \begin{array}{l} 511 \\ 473 \end{array} \right\} 984 \times 10^3 \text{ cells/mL} \times 2$  <sup>dilution factor</sup>

$1968 \times 10^3 \text{ cells/mL}$

⊕ 5 dish or 1.5 mL ←  $2.95 \times 10^6 \text{ cells}$

PLATED: 100 μL →  $197 \times 10^3 \text{ cells}$

100 μL + 100 μL →  $394 \times 10^3 \text{ cells}$

JUNE 14, 2002

HEMOCYTOMETER COUNTING

0Gy\* / 0Gy:  $\left. \begin{array}{l} 180 \\ 167 \end{array} \right\} 347$  }  $\left. \begin{array}{l} 183 \\ 192 \end{array} \right\} 375$  }  $\frac{722}{2} = 361 \times 10^3 \text{ cells/mL}$   
 + 0.5 mL →  $542 \times 10^3 \text{ cells}$

0Gy\* / 0.5Gy:  $\left. \begin{array}{l} 135 \\ 167 \end{array} \right\} 302$  }  $\left. \begin{array}{l} 165 \\ 141 \end{array} \right\} 306$  }  $\frac{608}{2} = 304 \times 10^3 \text{ cells/mL}$   
 + 0.5 mL →  $456 \times 10^3 \text{ cells}$

0Gy\* / 1Gy:  $\left. \begin{array}{l} 168 \\ 170 \end{array} \right\} 338$  }  $\left. \begin{array}{l} 165 \\ 153 \end{array} \right\} 318$  }  $\frac{656}{2} = 328 \times 10^3 \text{ cells/mL}$   
 + 0.5 mL →  $492 \times 10^3 \text{ cells}$

0Gy\* / 5Gy:  $\left. \begin{array}{l} 118 \\ 114 \end{array} \right\} 232$  }  $\left. \begin{array}{l} 120 \\ 106 \end{array} \right\} 226$  }  $\frac{458}{2} = 229 \times 10^3 \text{ cells/mL}$   
 + 0.5 mL →  $344 \times 10^3 \text{ cells}$

0 Gy\* / 10 Gy:  $\left. \begin{array}{l} 140 \\ 145 \\ 134 \\ 120 \end{array} \right\} \begin{array}{l} 285 \\ 254 \end{array} \right\} \frac{539}{2} = 270 \times 10^3 \text{ cells/mL}$   
 $+ 0.5 \text{ mL} \rightarrow 405 \times 10^3 \text{ cells}$

0 Gy\* / 20 Gy:  $\left. \begin{array}{l} 122 \\ 117 \\ 115 \\ 107 \end{array} \right\} \begin{array}{l} 239 \\ 222 \end{array} \right\} \frac{461}{2} = 230 \times 10^3 \text{ cells/mL}$   
 $+ 0.5 \text{ mL} \rightarrow 347 \times 10^3 \text{ cells}$

---

0 Gy\* + 5 Gy:  $\left. \begin{array}{l} 120 \\ 147 \\ 121 \\ 154 \end{array} \right\} \begin{array}{l} 267 \\ 275 \end{array} \right\} \frac{542}{2} = 271 \times 10^3 \text{ cells/mL}$   
 $+ 0.5 \text{ mL} \rightarrow 407 \times 10^3 \text{ cells}$

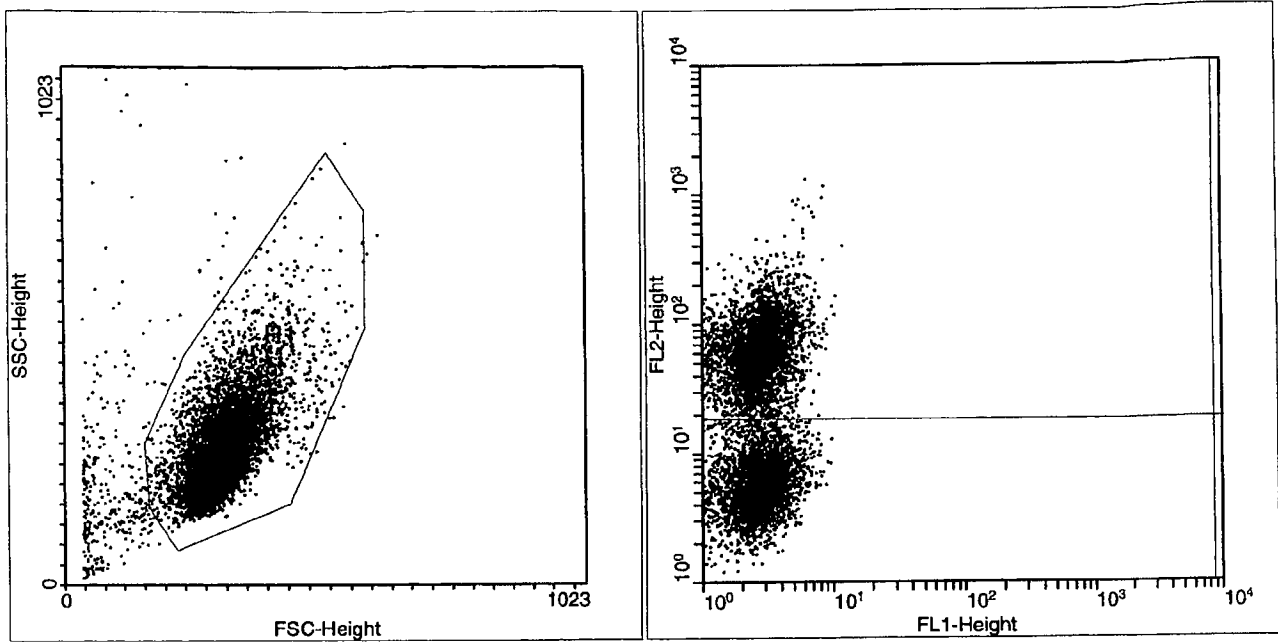
0 Gy\* + 10 Gy:  $\left. \begin{array}{l} 124 \\ 118 \\ 119 \\ 110 \end{array} \right\} \begin{array}{l} 242 \\ 229 \end{array} \right\} \frac{471}{2} = 236 \times 10^3 \text{ cells/mL}$   
 $+ 0.5 \text{ mL} \rightarrow 354 \times 10^3 \text{ cells}$

0 Gy\* + 20 Gy:  $\left. \begin{array}{l} 88 \\ 105 \\ 107 \\ 107 \end{array} \right\} \begin{array}{l} 193 \\ 214 \end{array} \right\} \frac{407}{2} = 203 \times 10^3 \text{ cells/mL}$   
 $+ 0.5 \text{ mL} \rightarrow 305 \times 10^3 \text{ cells}$

Old dye lot.

5ml dye





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 16:01:20 2002

Gates: R1

Project: Experiment:

File: 061402 Sample: 0Gy(DiI)/0Gy

Date: 14-Jun-2 Parameters: 7

Total Events 10000 Gated Events 9513 95.13%

System: Log Parameter Means: Geometric

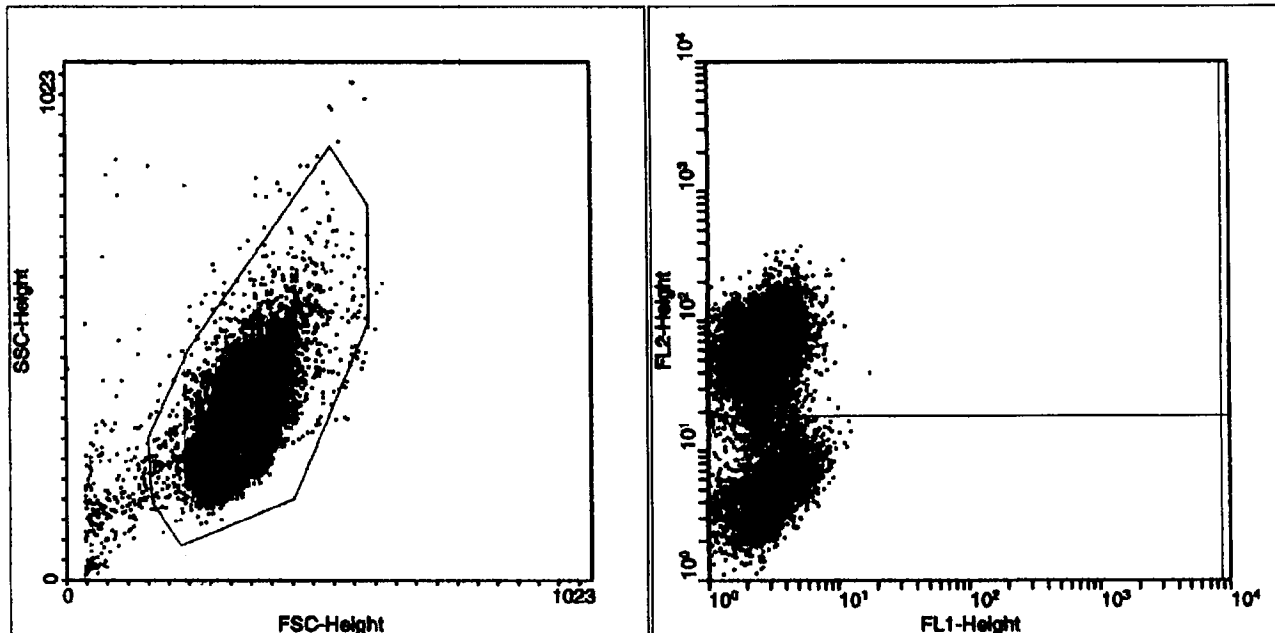
Quad Stats

FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 1008,324

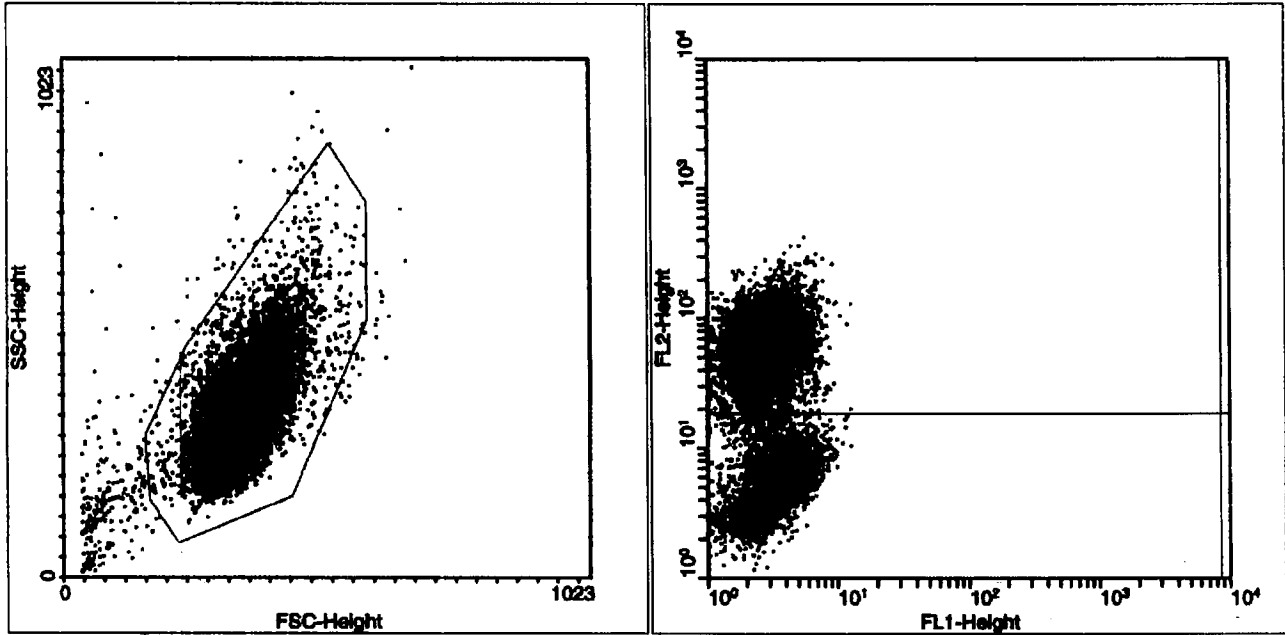
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.7	63.2	4900	49.00	51.51
2 UR	0.0	0.0	0	0.00	0.00
3 LL	2.7	5.7	4613	46.13	48.49
4 LR	0.0	0.0	0	0.00	0.00

= 1.0%



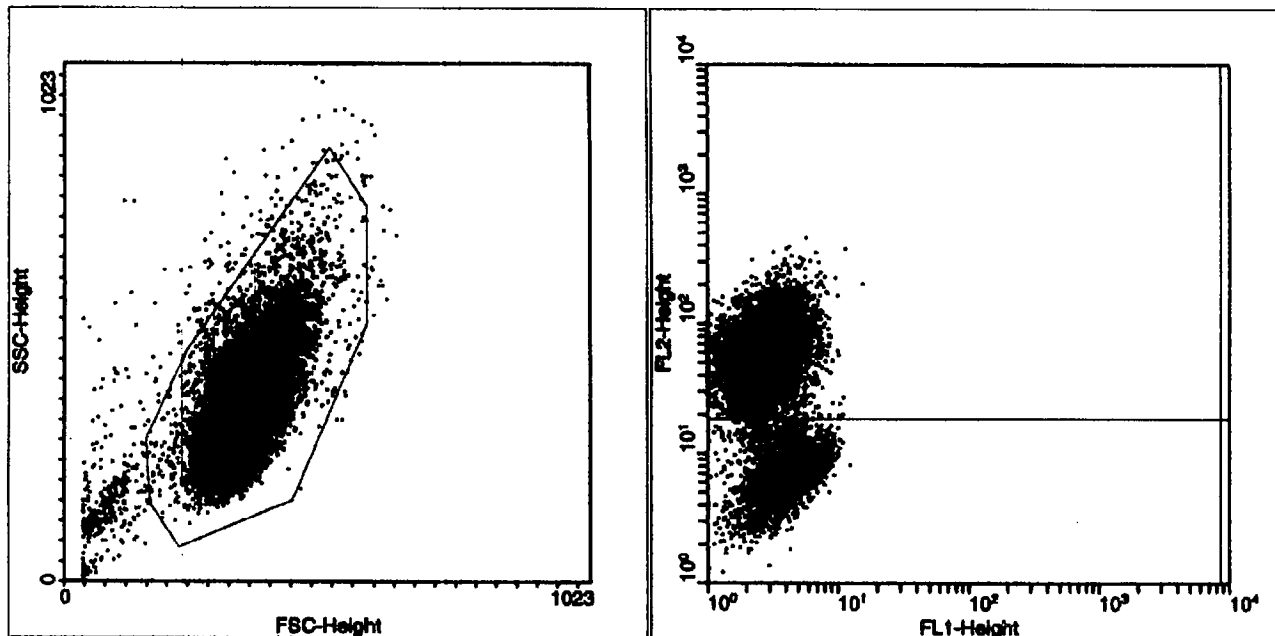
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 16:02:10 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061402 Sample: 0Gy(DIT)/0.5Gy  
 Date: 14-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9603 96.03%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,324

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.8	62.5	5313	53.13	<u>55.33</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.1	5.1	4290	42.90	<u>44.67</u>
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 16:02:52 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061402 Sample: 0Gy(DiI)/1Gy  
 Date: 14-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9595 95.95%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,324

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.9	61.6	5694	56.94	59.34
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.3	5.5	3901	39.01	40.66
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 16:03:24 2002

Gates: R1

Project: Experiment:

File: 061402 Sample: 0Gy(DI1)/5Gy

Date: 14-Jun-2 Parameters: 7

Total Events 10000 Gated Events 9431 94.31%

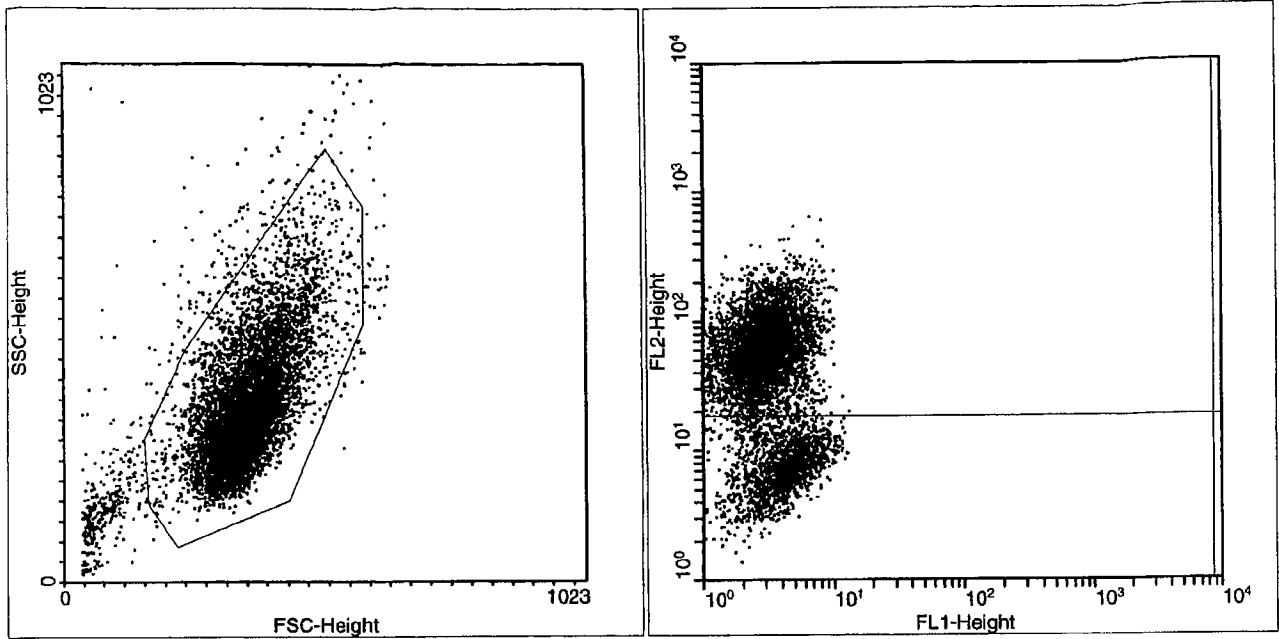
System: Log Parameter Means: Geometric

Quad Stats

FL1-Height (Log) vs FL2-Height (Log)

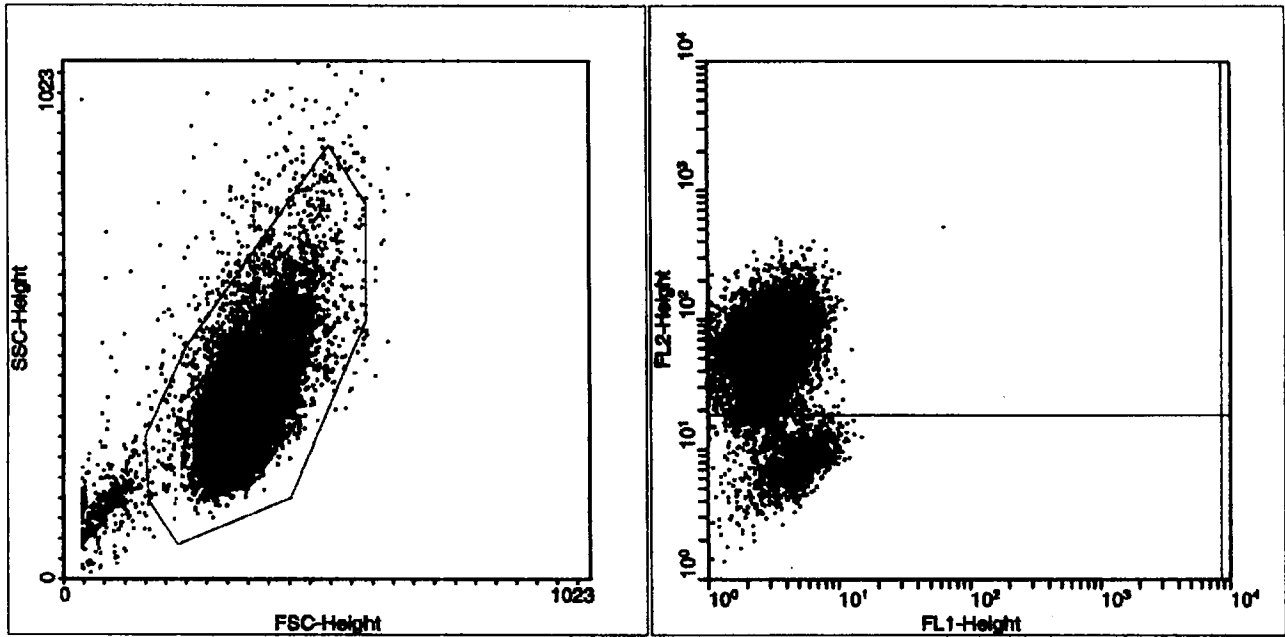
Quadrant x,y: 1008,324

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.9	61.6	6561	65.61	<u>69.57</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.8	6.6	2870	28.70	<u>30.43</u>
4 LR	0.0	0.0	0	0.00	0.00



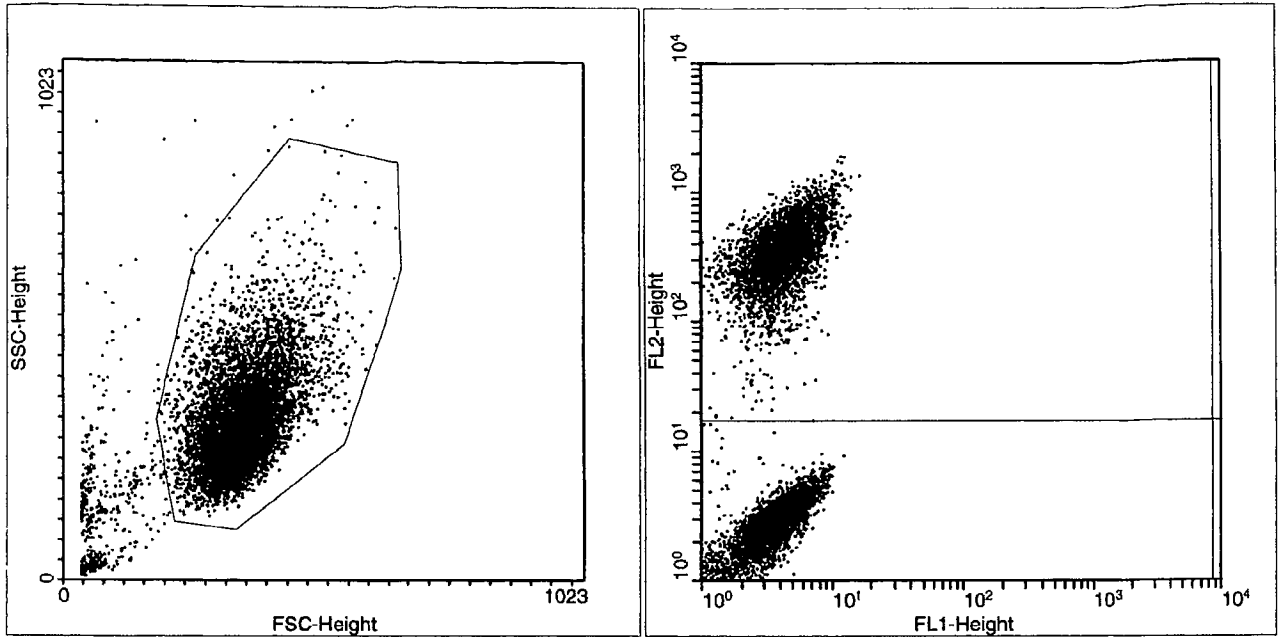
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 16:03:58 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061402 Sample: OGy(DiI)/10Gy  
 Date: 14-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9299 92.99%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,324

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.9	63.4	6943	69.43	<u>74.66</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.0	7.5	2356	23.56	<u>25.34</u>
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 16:04:34 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061402 Sample: 00y(DII)/20Gy  
 Date: 14-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9166 91.66%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,324

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	2.9	62.7	7522	75.22	<u>82.06</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.0	8.5	1644	16.44	<u>17.93</u>
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 15:44:53 2002

Gates: R1

Project: Experiment:

File: 061402 Sample: 0Gy/0Gy (DiI) + 0Gy/0.5Gy

Date: 14-Jun-2 Parameters: 7

Total Events 10000 Gated Events 9364 93.64%

System: Log Parameter Means: Geometric

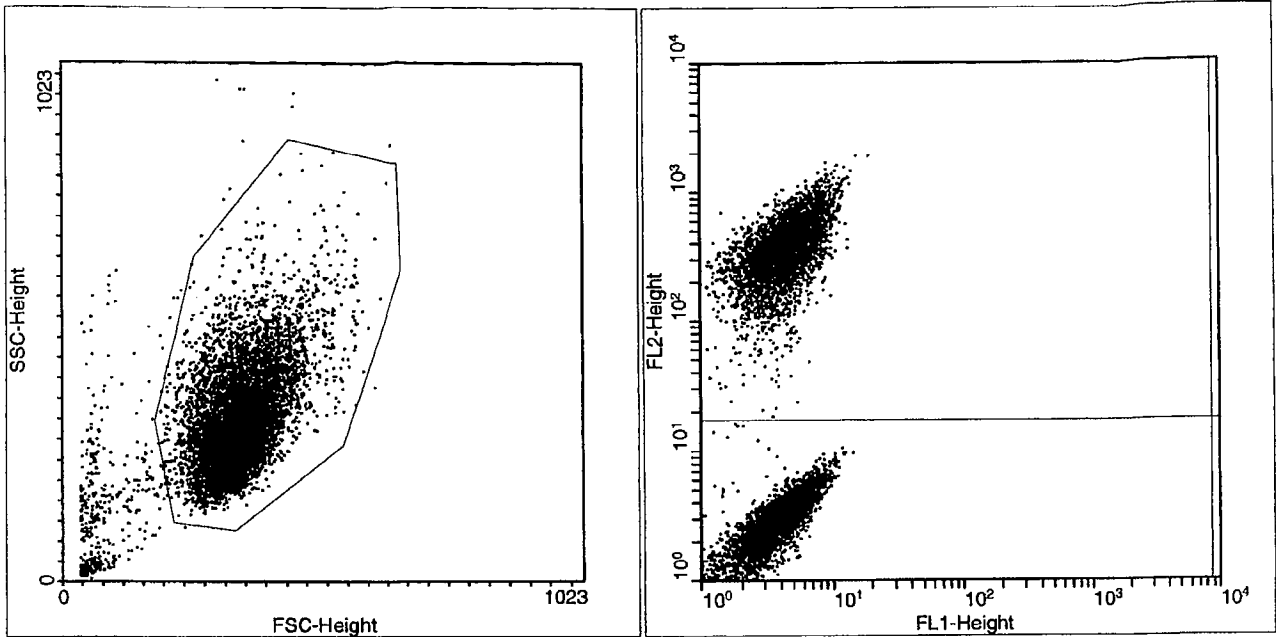
Quad Stats

FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 1008,316

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.2	340.0	4753	47.53	<u>50.76</u>
2 UR	0.0	0.0	0	0.00	<u>0.00</u>
3 LL	3.4	2.6	4611	46.11	<u>49.24</u>
4 LR	0.0	0.0	0	0.00	0.00

1.03

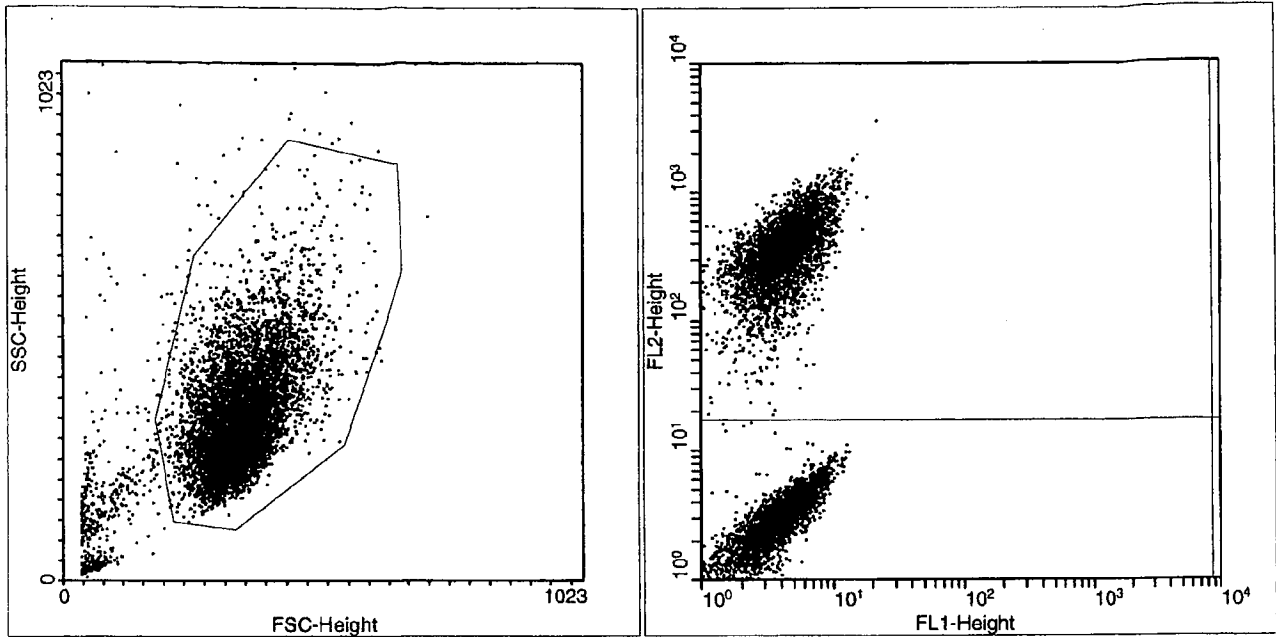


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 15:46:36 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061402 Sample: 0Gy/0Gy (DiI)+0Gy/1Gy  
 Date: 14-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9438 94.38%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,316

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.3	346.2	4925	49.25	<del>52.18</del>
2 UR	0.0	0.0	0	0.00	<del>0.00</del>
3 LL	3.5	2.7	4513	45.13	<del>47.82</del>
4 LR	0.0	0.0	0	0.00	0.00

-1.09



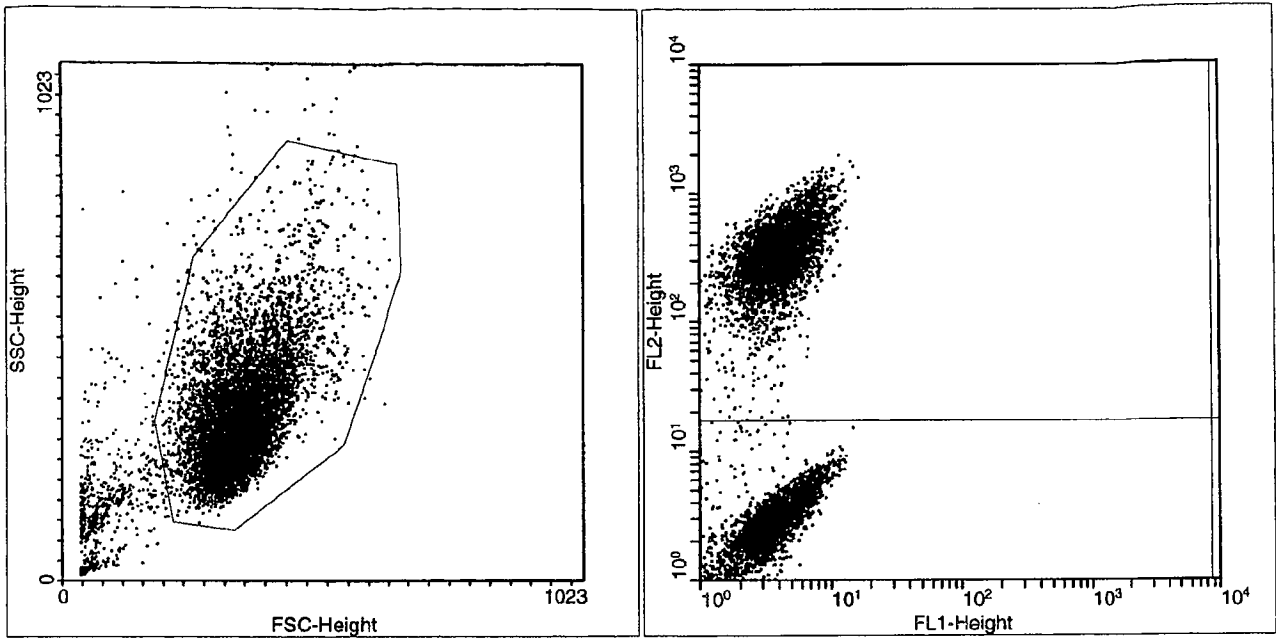


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 15:48:25 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061402 Sample: 0Gy/0Gy (Di I) + 0Gy/5Gy  
 Date: 14-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9310 93.10%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,316

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.3	343.2	5180	51.80	55.64
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.7	2.9	4130	41.30	44.36
4 LR	0.0	0.0	0	0.00	0.00

= 1.25



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 15:49:10 2002

Gates: R1

Project: Experiment:  
 File: 061402 Sample: 0Gy/0Gy (DiI)+0Gy/10Gy

Date: 14-Jun-2 Parameters: 7

Total Events 10000 Gated Events 9228 92.28%

System: Log Parameter Means: Geometric

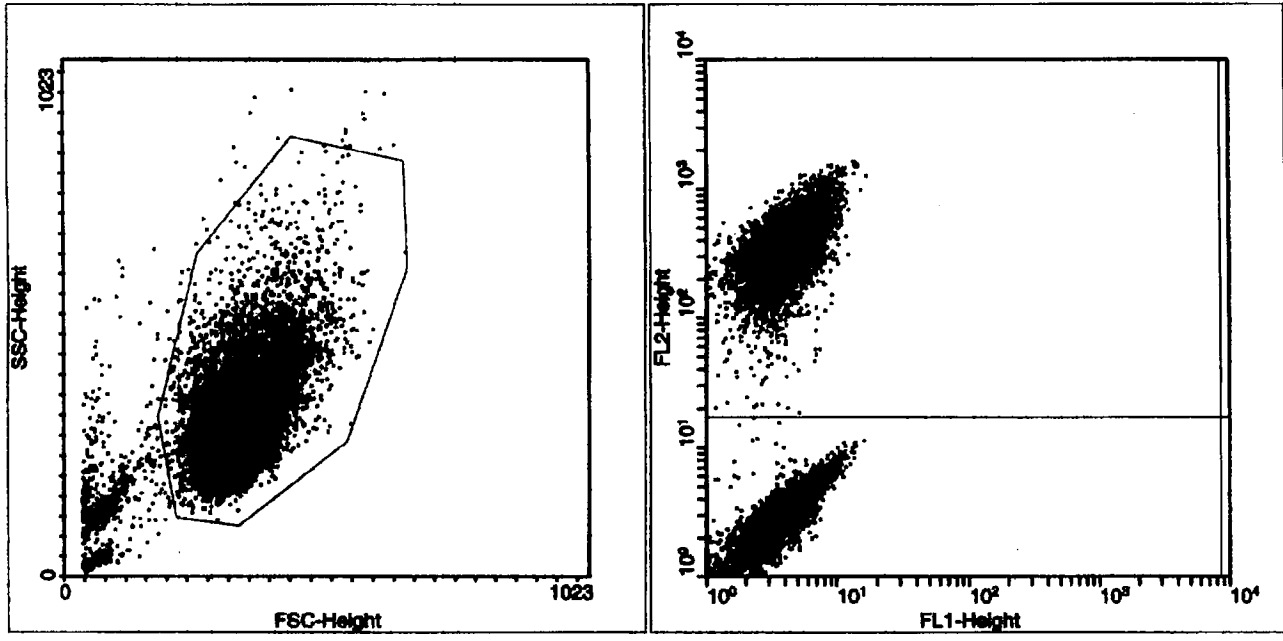
Quad Stats

FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 1008,316

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.1	340.5	5339	53.39	57.86
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.6	2.9	3889	38.89	42.14
4 LR	0.0	0.0	0	0.00	0.00

= 1.37



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 15:49:55 2002

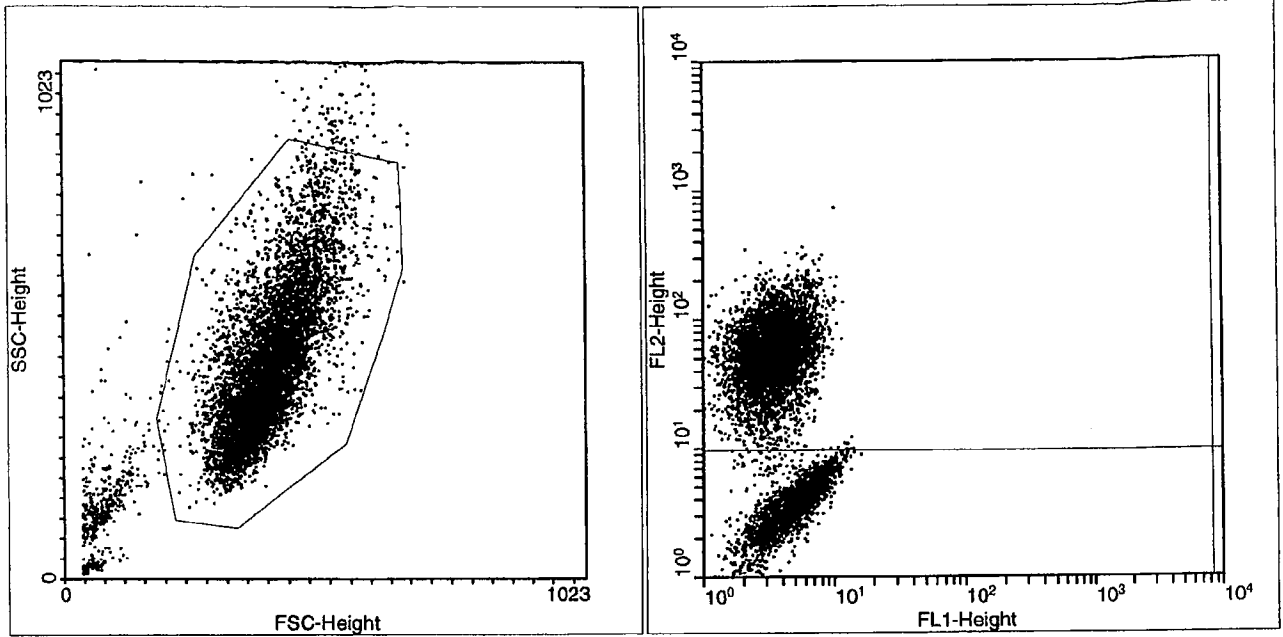
Gates: R1  
 Project: Experiment:  
 File: 061402 Sample: 0Gy/0Gy(D1I)+0Gy/20Gy  
 Date: 14-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9151 91.51%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 1008,316

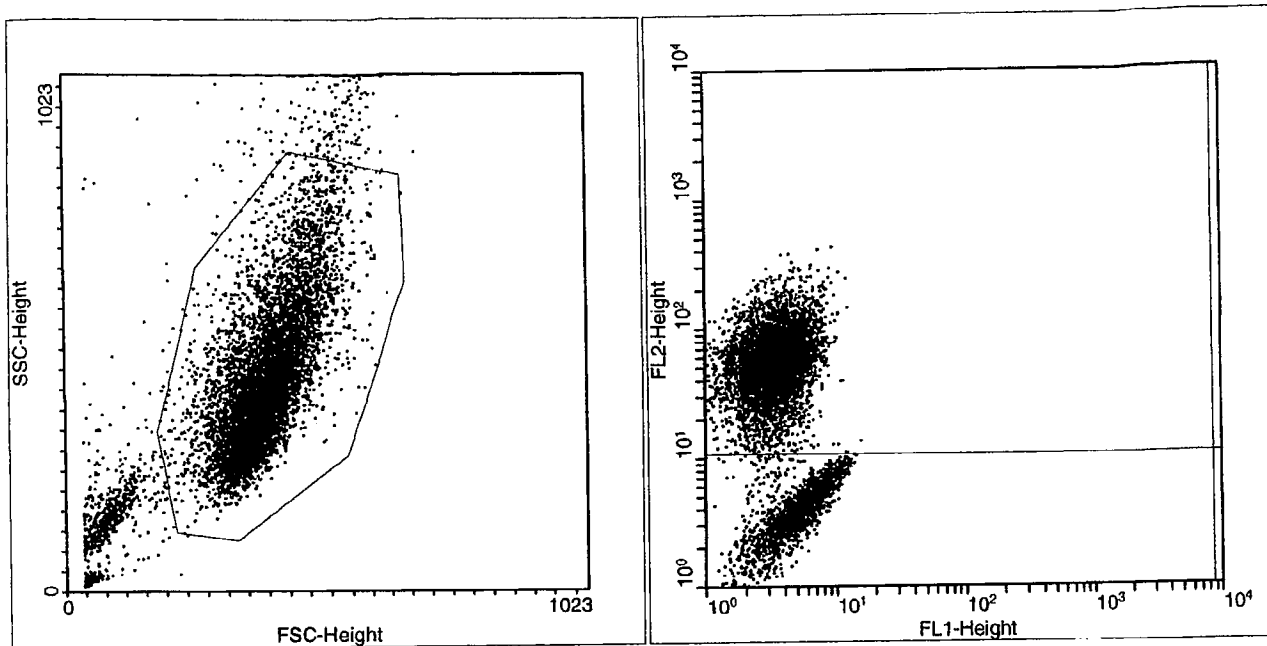
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.1	345.0	5388	53.88	<u>58.88</u>
2 UR	0.0	0.0	0	0.00	<del>0.00</del>
3 LL	3.6	2.8	3763	37.63	<u>41.12</u>
4 LR	0.0	0.0	0	0.00	0.00

1.43



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 15:51:21 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061402 Sample: 0Gy(DiI)+5Gy  
 Date: 14-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9242 92.42%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1004,252

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.4	55.7	6386	63.86	69.10
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.7	3.6	2856	28.56	30.90
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 15:53:04 2002

Gates: R1

Project: Experiment: 0Gy(DiI)+10Gy

File: 061402 Sample: 0Gy(DiI)+10Gy

Date: 14-Jun-2 Parameters: 7

Total Events 10000 Gated Events 8825 88.25%

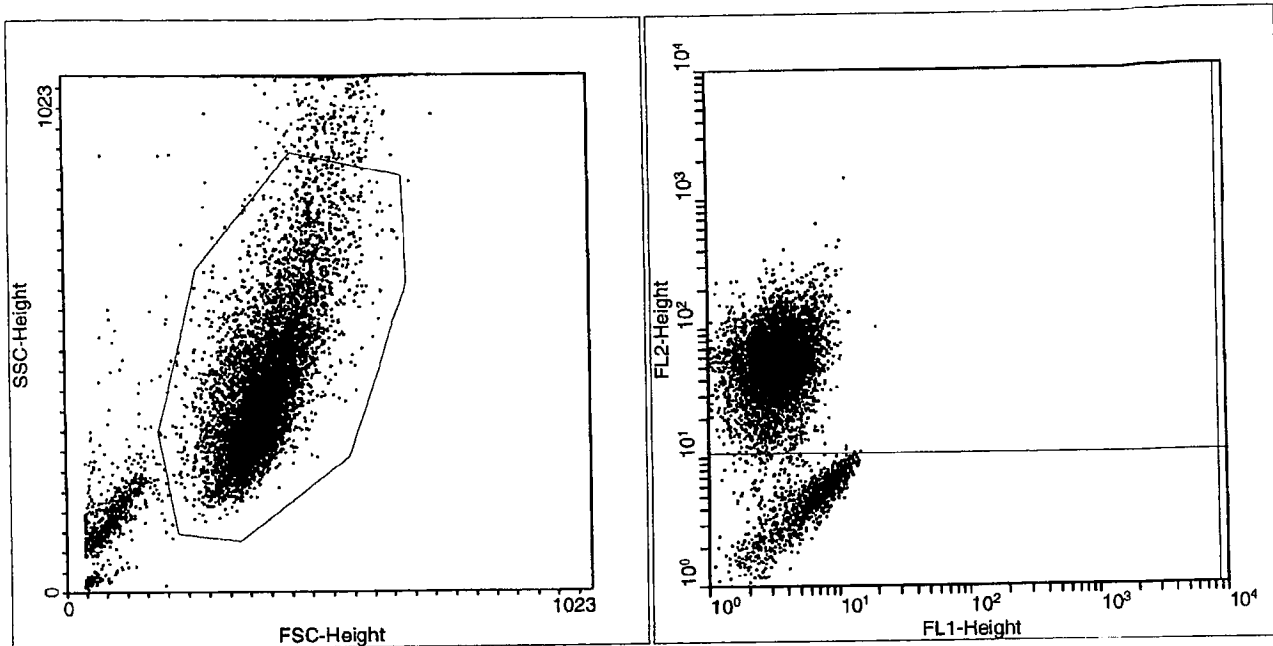
System: Log Parameter Means: Geometric

Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

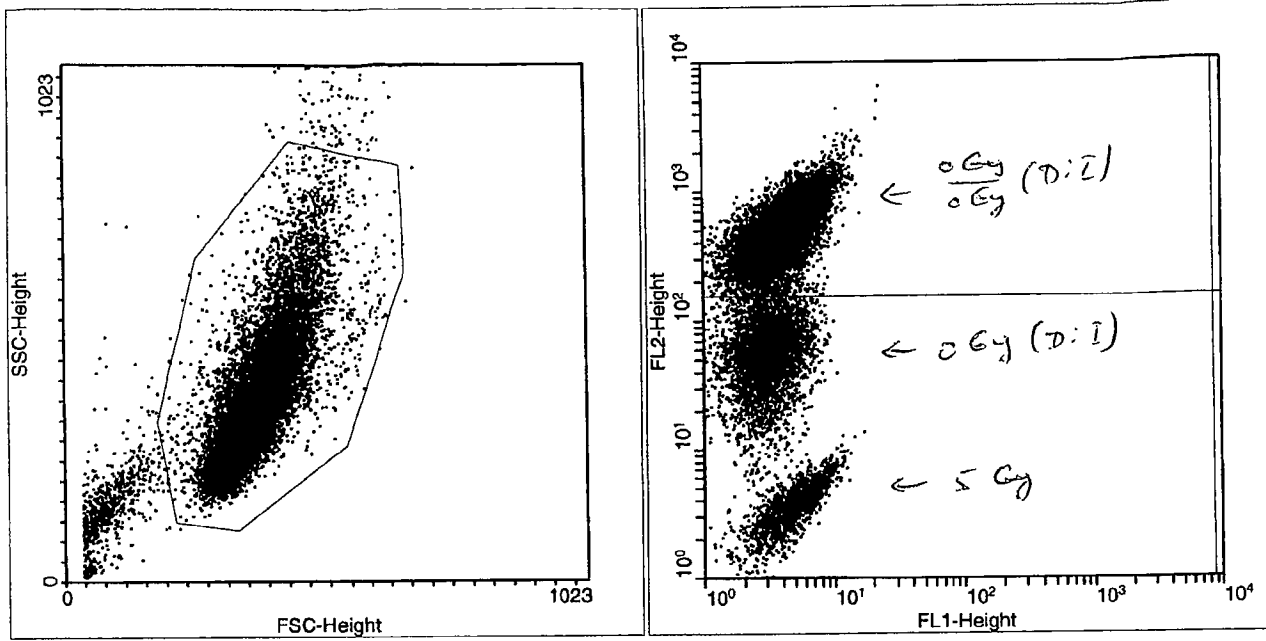
Quadrant x,y: 1008,264

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.3	55.7	6531	65.31	74.01
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.8	3.8	2294	22.94	25.99
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 15:53:43 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061402 Sample: 0Gy(DiI)+20Gy  
 Date: 14-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8553 85.53%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,264

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.4	55.5	7195	71.95	84.12
2 UR	0.0	0.0	0	0.00	0.00
3 LL	5.3	4.5	1358	13.58	15.88
4 LR	0.0	0.0	0	0.00	0.00

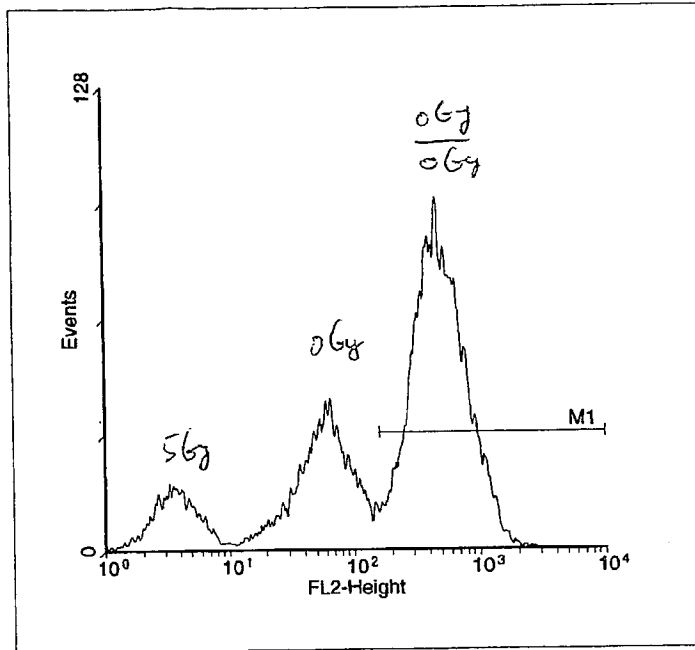


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 15:57:50 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061402 Sample: 0Gy(DiI)+5Gy+0Gy/0Gy(DiI)  
 Date: 14-Jun-2 Parameters: 7  
 Total Events 20000 Gated Events 18917 94.58%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,560

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.0	482.5	11735	58.68	62.03
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.4	25.2	7182	35.91	37.97
4 LR	0.0	0.0	0	0.00	0.00

= 1.63



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sun Jun 16 12:40:21 2002  
 Gates: R1

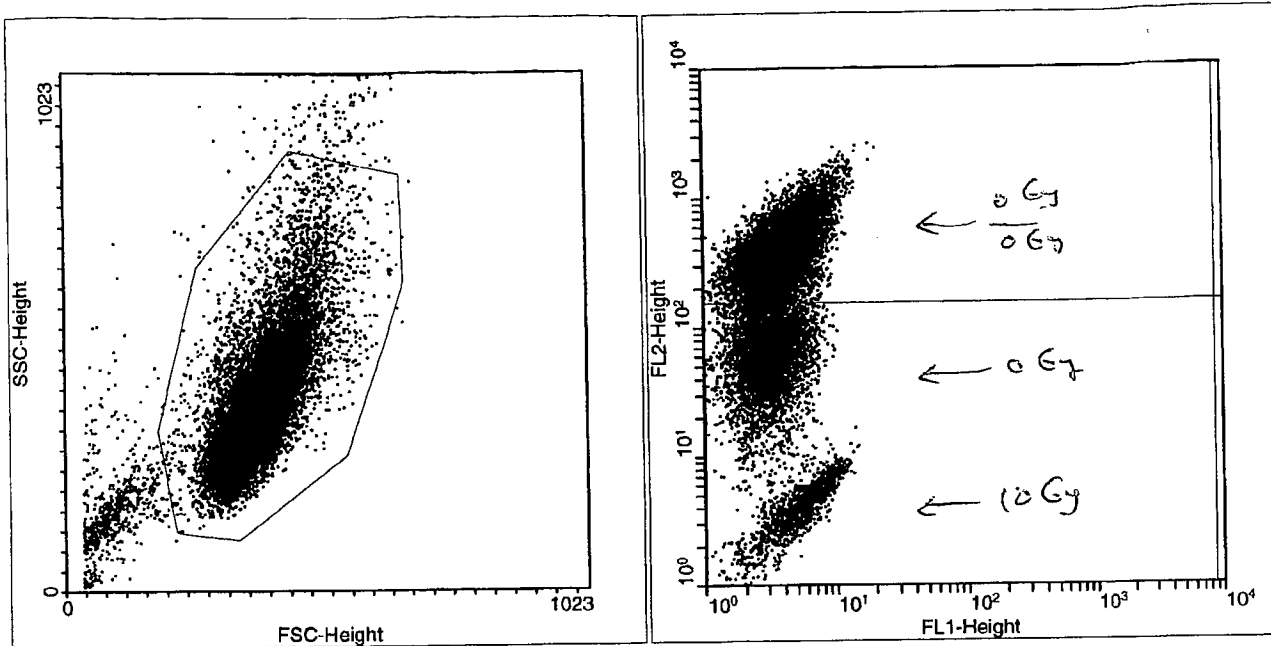
Project: Experiment:  
 File: 061402 Sample: 0Gy(DiI)+5Gy+0Gy/0Gy(DiI)  
 Date: 14-Jun-2 Parameters: 1

Total Events 20000 Gated Events 18716 93.58%  
 System: Log Parameter Means: Geometric

Param name	M	Low,High	Events	%Total	%Gated	GMean	CV	Peak,Value
FL2-Heigh	0	0,1023	18716	93.58	100.00	158.90	33.26	106,449.101
	1	564,1023	11614	58.07	62.05	483.40	7.80	106,449.101

37.95  
 1.64





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Sat Jun 15 15:58:18 2002

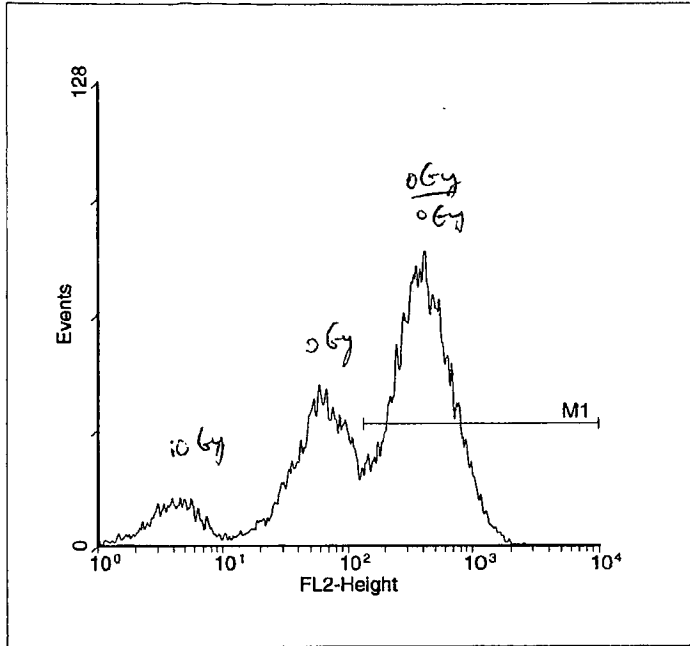
Gates: R1  
 Project: Experiment:  
 File: 061402 Sample: 0Gy (DiI)+10Gy+0Gy/0Gy (DiI)  
 Date: 14-Jun-2 Parameters: 7  
 Total Events 20000 Gated Events 18900 94.50%

System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,560

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.1	418.5	10993	54.97	58.16
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.4	32.6	7907	39.54	41.84
4 LR	0.0	0.0	0	0.00	0.00

(1.39)

CORRECTED



Multiple Document Interface for Flow Cytometry  
WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
Sun Jun 16 12:42:15 2002  
Gates: R1

Project: Experiment:  
File: 061402 Sample: 0Gy (DiI) + 10Gy + 0Gy / 0Gy (DiI)

Date: 14-Jun-2 Parameters: 1  
Total Events 20000 Gated Events 18658 93.29%

System: Log Parameter Means: Geometric

Param name	M	Low, High	Events	%Total	%Gated	GMean	CV	Peak, Value
FL2-Heigh	0	0, 1023	18658	93.29	100.00	146.86	30.54	90,421.697
	1	544, 1023	11278	56.39	60.45	404.15	8.87	90,421.697

39.55  
1.53

JUNE 16, 2002

CALCULATIONS ( MICROSCOPY )

① AMOUNTS OF UNIRRADIATED CELLS IN THE CO-CULTURE WITH IRRADIATED CELLS

$\frac{0.6\text{Gy}^* / 0.6\text{Gy}}{100\%} = \frac{542 \times 10^3 \times 51.5\%}{100\%} = 279 \times 10^3 \text{ cells}$

$\frac{0.6\text{Gy}^* / 0.5\text{Gy}}{100\%} = \frac{456 \times 10^3 \times 55.3\%}{100\%} = 252 \times 10^3 \text{ cells}$

$\frac{0.6\text{Gy}^* / 1.6\text{Gy}}{100\%} = \frac{492 \times 10^3 \times 59.3\%}{100\%} = 292 \times 10^3 \text{ cells}$

$\frac{0.6\text{Gy}^* / 5.6\text{Gy}}{100\%} = \frac{344 \times 10^3 \times 69.6\%}{100\%} = 239 \times 10^3 \text{ cells}$

$\frac{0.6\text{Gy}^* / 10.6\text{Gy}}{100\%} = \frac{405 \times 10^3 \times 74.7\%}{100\%} = 303 \times 10^3 \text{ cells}$

$\frac{0.6\text{Gy}^* / 20.6\text{Gy}}{100\%} = \frac{347 \times 10^3 \times 82.1\%}{100\%} = 285 \times 10^3 \text{ cells}$

② AMOUNTS OF IRRADIATED CELLS IN THE CO-CULTURE WITH UNIRRADIATED CELLS

$\frac{0.6\text{Gy}^* / 0.6\text{Gy}}{100\%} = \frac{542 \times 10^3 \times 48.5\%}{100\%} = 263 \times 10^3 \text{ cells}$

$\frac{0.6\text{Gy}^* / 0.5\text{Gy}}{100\%} = \frac{456 \times 10^3 \times 44.7\%}{100\%} = 204 \times 10^3 \text{ cells}$

$\frac{0.6\text{Gy}^* / 1.6\text{Gy}}{100\%} = \frac{492 \times 10^3 \times 40.7\%}{100\%} = 200 \times 10^3 \text{ cells}$

$\frac{0.6\text{Gy}^* / 5.6\text{Gy}}{100\%} = \frac{344 \times 10^3 \times 30.4\%}{100\%} = 105 \times 10^3 \text{ cells}$

$$0.6\text{Gy}^* / (10.6\text{Gy}) : \frac{405 \times 10^3 \times 25.3\%}{100\%} = 102 \times 10^3 \text{ cells}$$

$$0.6\text{Gy} / (20.6\text{Gy}) : \frac{347 \times 10^3 \times 17.9\%}{100\%} = 62 \times 10^3 \text{ cells}$$

3) AMOUNTS OF UNIRRADIATED AND IRRADIATED CELLS CULTURED SEPARATELY

$$0.6\text{Gy}^* + 5.6\text{Gy} : \frac{407 \times 10^3 \times 69.1\%}{100\%} = 281 \times 10^3 \text{ cells}$$

$$0.6\text{Gy}^* + (5.6\text{Gy}) : \frac{407 \times 10^3 \times 30.9\%}{100\%} = 126 \times 10^3 \text{ cells}$$

$$0.6\text{Gy}^* + 10.6\text{Gy} : \frac{354 \times 10^3 \times 74.0\%}{100\%} = 262 \times 10^3 \text{ cells}$$

$$0.6\text{Gy}^* + (10.6\text{Gy}) : \frac{354 \times 10^3 \times 26.0\%}{100\%} = 92 \times 10^3 \text{ cells}$$

$$0.6\text{Gy}^* + 20.6\text{Gy} : \frac{305 \times 10^3 \times 84.1\%}{100\%} = 257 \times 10^3 \text{ cells}$$

$$0.6\text{Gy}^* + (20.6\text{Gy}) : \frac{305 \times 10^3 \times 15.9\%}{100\%} = 48 \times 10^3 \text{ cells}$$

CALCULATIONS (FCM)

$$\textcircled{1} \frac{0.6\text{Gy}(X_1) + 0.6\text{Gy}(Y_1)}{0.6\text{Gy}(X_2) + 0.5\text{Gy}(Y_2)} = \frac{50.76}{49.24} = 1.03$$

$$X_1(51.5) + Y_1(48.5) = 100 \text{ events} \quad - \text{ CONTROL}$$

$$\frac{100}{0.6\text{Gy}(X_2) + 0.5\text{Gy}(Y_2)} = 1.03$$

$$0.6\text{Gy}(X_2) + 0.5\text{Gy}(Y_2) = 97.1$$

$$\text{CONVERTED } X_2 = \frac{97.1 \times 55.3\%}{100\%} = \overset{\text{events}}{53.7} \rightarrow 0.6\text{Gy}$$

$$\text{CONVERTED } Y_2 = \frac{97.1 \times 44.7\%}{100\%} = 43.4 \rightarrow 0.5\text{Gy}$$

$$\textcircled{2} \frac{55.33}{44.67} = 1.24 \quad \frac{52.18}{47.82} = 1.09 \quad \frac{100}{1.09} = 91.7$$

$$\text{CONVERTED } X_2 = \frac{91.7 \times 59.3\%}{100\%} = 54.4 \rightarrow 0.6\text{Gy}$$

$$\text{CONVERTED } Y_2 = \frac{91.7 \times 40.7\%}{100\%} = 37.3 \rightarrow 1.6\text{Gy}$$

$$\textcircled{3} \frac{55.64}{44.36} = 1.25 \quad \frac{100}{1.25} = 80$$

$$\text{CONVERTED } X_2 = \frac{80 \times 69.6\%}{100\%} = 55.7 \rightarrow 0.6\text{Gy}$$

$$\text{CONVERTED } Y_2 = \frac{80 \times 30.4\%}{100\%} = 24.3 \rightarrow 5\text{Gy}$$

$$\textcircled{4} \quad \frac{57.86}{42.14} = 1.37 \quad \frac{100}{1.37} = 73$$

$$\text{CONVERTED } X_2 = \frac{73 \times 74.7\%}{100\%} = 54.5 \longrightarrow 0 \text{ Gy}$$

$$\text{CONVERTED } Y_2 = \frac{73 \times 25.3\%}{100\%} = 18.5 \longrightarrow 10 \text{ Gy}$$

$$\textcircled{5} \quad \frac{58.88}{41.12} = 1.43 \quad \frac{100}{1.43} = 69.9$$

$$\text{CONVERTED } X_2 = \frac{69.9 \times 82.1\%}{100\%} = 57.4 \longrightarrow 0 \text{ Gy}$$

$$\text{CONVERTED } Y_2 = \frac{69.9 \times 17.9\%}{100\%} = 12.5 \longrightarrow 20 \text{ Gy}$$

%U Exp 1	%I Exp 1	%U Exp 2	%I Exp 2	%U Exp 3	%I Exp 3	%U Exp 4	%I Exp 4	Scheme
48.57	51.43	49.36	50.64	51.38	48.62	51.51	48.49	0*/0
54.36	45.65	55.23	44.77	56.45	43.55	55.33	44.67	0*/0.5
59.25	40.75	59.18	40.82	59.17	40.83	59.34	40.66	0*/1
74.26	25.74	70.8	29.2	71.6	28.4	69.57	30.43	0*/5
82.6	17.4	75.83	24.17	77.05	22.95	74.66	25.34	0*/10
86.89	13.11	81.83	18.17	81.85	18.15	82.06	17.84	0*/20

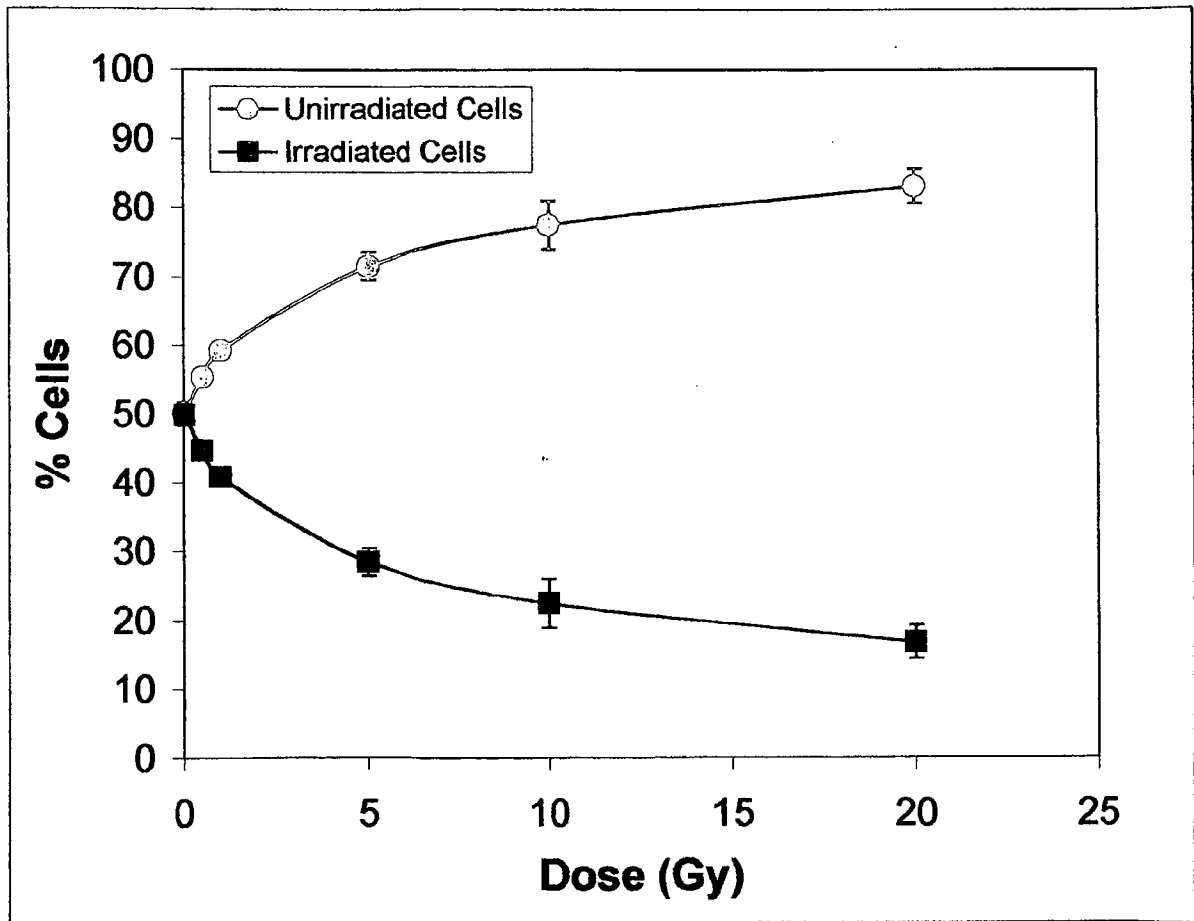
①

$$\frac{A^2 + B^2 + C^2 + D^2}{2(A+B)}$$

① ② ③

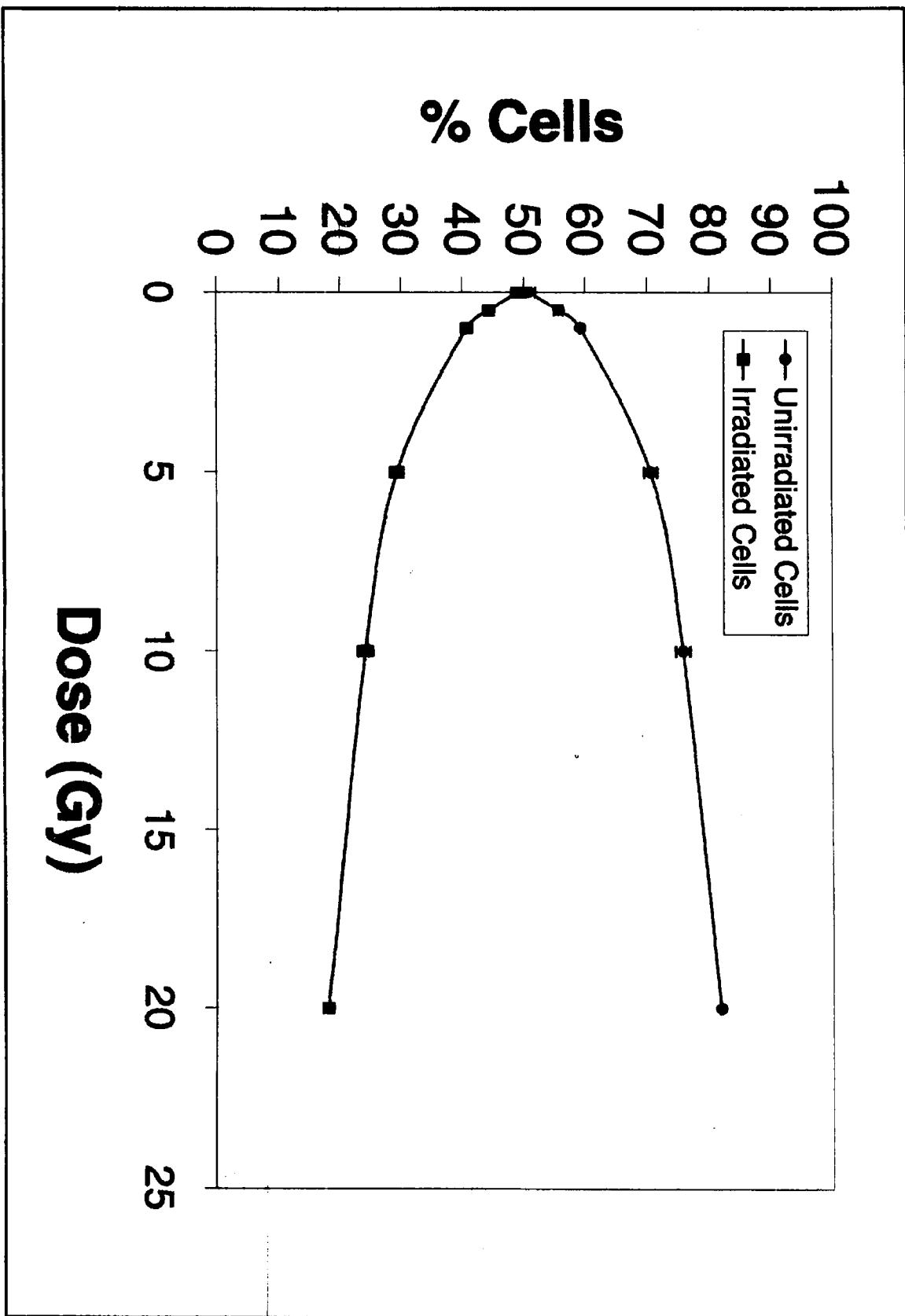
$$\frac{ABC}{2} \pm \sqrt{ABC} + \sqrt{B-ABC} + \sqrt{C-ABC}$$

Dose (Gy)	Unirradiated Cells	Irradiated Cells	SD %U	SD %I
0	50.205	49.795	1.468662	1.468662
0.5	55.34	44.66	0.861085	0.861085
1	59.235	40.765	0.078528	0.078528
5	71.5575	28.4425	1.985722	1.985722
10	77.535	22.465	3.51483	3.51483
20	83.1575	16.8175	2.490507	2.476279

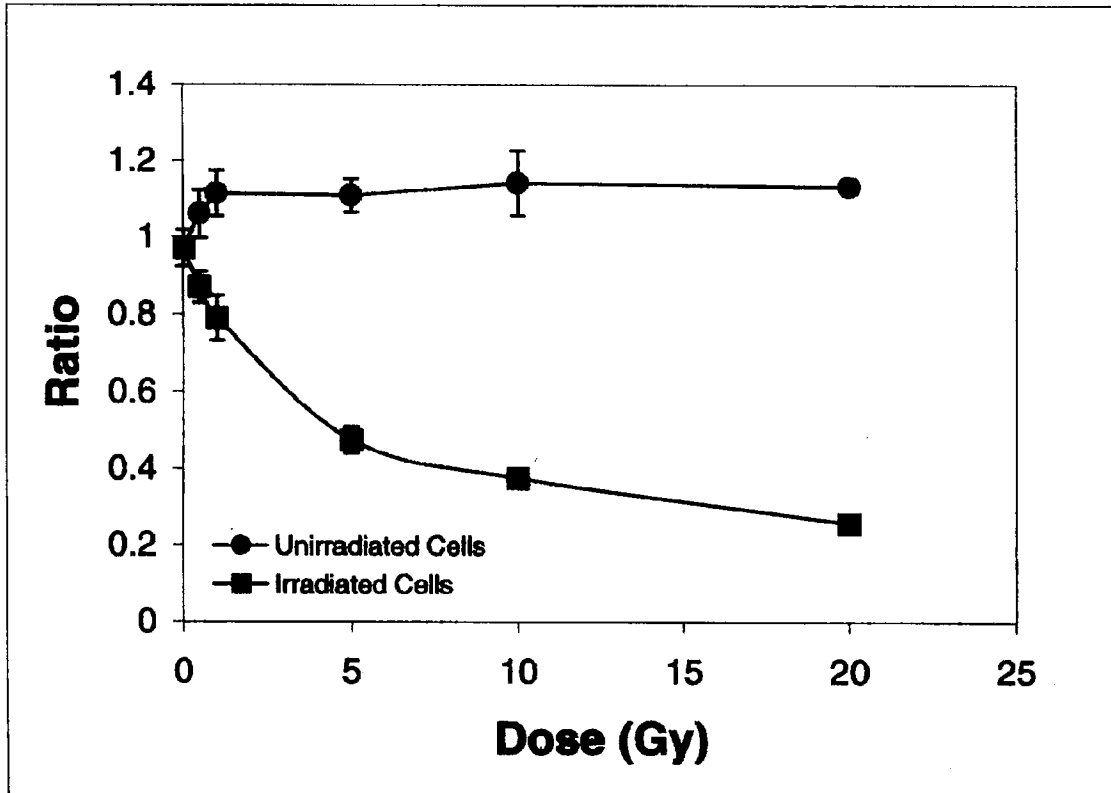




*Experiments # 2, 3, 4.*



Dose (Gy)	X (Exp 2)	Y (Exp 2)	X (Exp 3)	Y (Exp 3)	X (Exp 4)	Y (Exp 4)	RU (Exp 2)	RU (Exp 3)
0	49.36	50.64	51.4	48.6	51.5	48.5	1.025932	0.945525
0.5	55.8	45.2	51.8	40	53.7	43.4	1.13047	1.007782
1	54.8	37.8	60.4	41.6	54.4	37.3	1.110211	1.175097
5	53.7	22.1	58.6	23.7	55.7	24.3	1.087925	1.159533
10	56.2	17.9	63.2	18.9	54.5	18.5	1.138574	1.229572
20	56.1	12.4	58.9	13.1	57.4	12.5	1.136548	1.145914



RU (Exp 4)	RU (Avg)	Std	RI (Exp 2)	RI (Exp 3)	RI (Exp 4)	RI (Avg)	Std
0.941748	0.971068	0.047551	1.025932	0.945525	0.941748	0.971068	0.047551
1.042718	1.060324	0.06321	0.892575	0.823045	0.894845	0.870155	0.040814
1.056311	1.113873	0.059478	0.746445	0.855967	0.769072	0.790495	0.057818
1.081553	1.109671	0.0433	0.436414	0.487654	0.501031	0.475033	0.034107
1.058252	1.142133	0.085715	0.353476	0.388889	0.381443	0.374603	0.018671
1.114563	1.132342	0.016093	0.244866	0.269547	0.257732	0.257382	0.012345

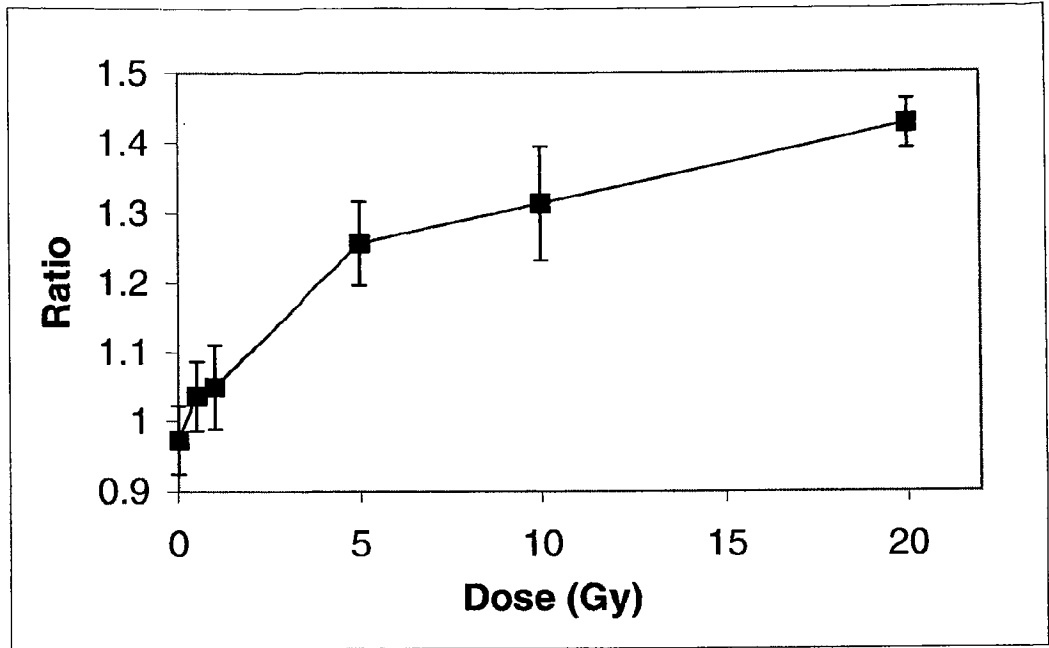


Ratio:  $\frac{0.6 \text{ Gy}}{0.6 \text{ Gy}}$ , where

(X) = 0.5; 1; 5; 10; 20 Gy

Dose (Gy)	Exp 2	Exp 3	Exp 4	Avg $\pm$ SD
0	1.03	0.95	0.94	0.973333 0.049329
0.5	0.99	1.09	1.03	1.036667 0.050332
1	1.08	0.98	1.09	1.05 0.060828
5	1.32	1.2	1.25	1.256667 0.060277
10	1.35	1.22	1.37	1.313333 0.081445
20	1.46	1.39	1.43	1.426667 0.035119

$4.2 \times 10^5$  cells



---

#5

BYSTANDER EFFECTS (β - IRRADIATION)

JUNE 17, 2002

WB/24 100% confluency

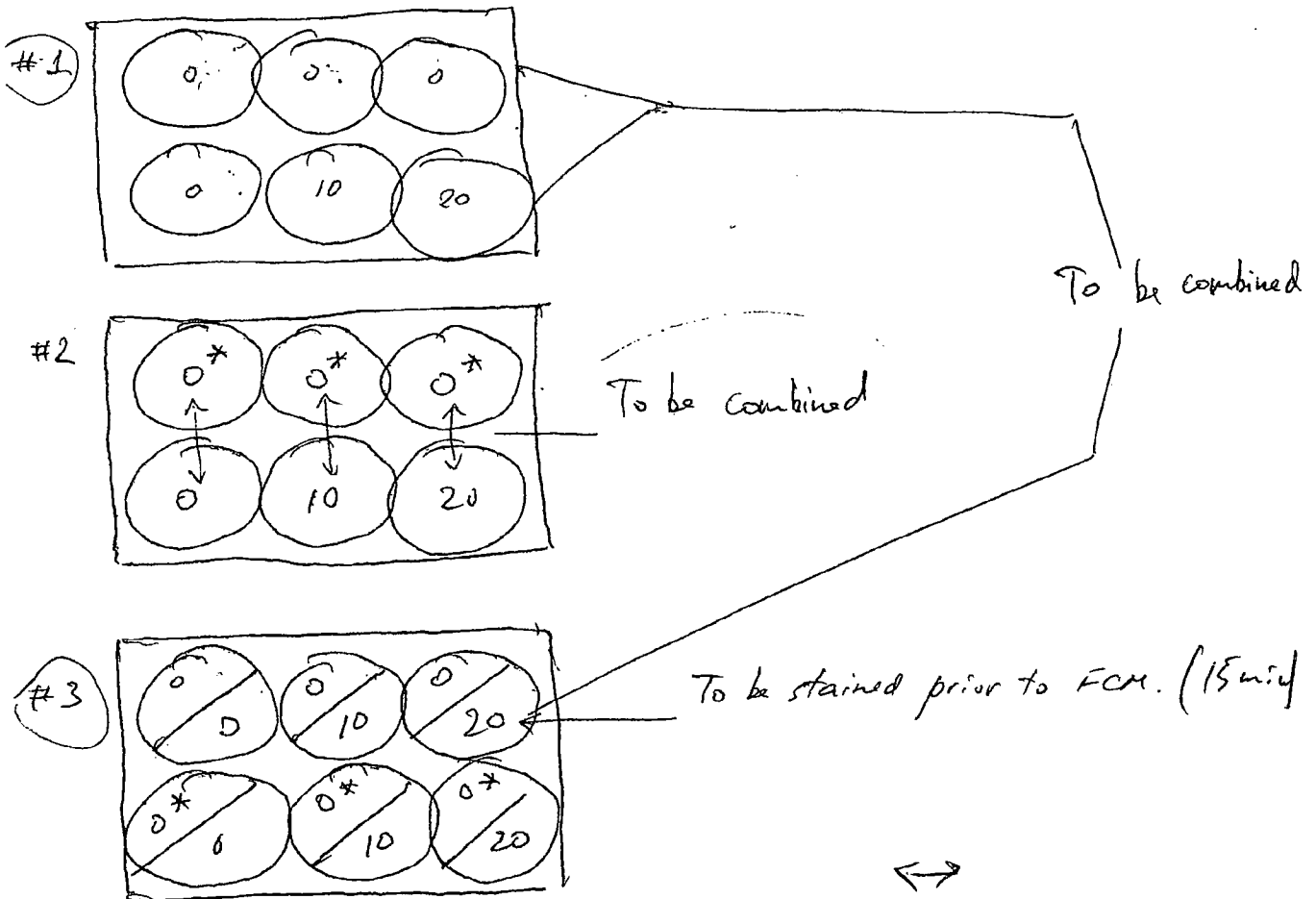
IRRADIATION TIME: 8:30

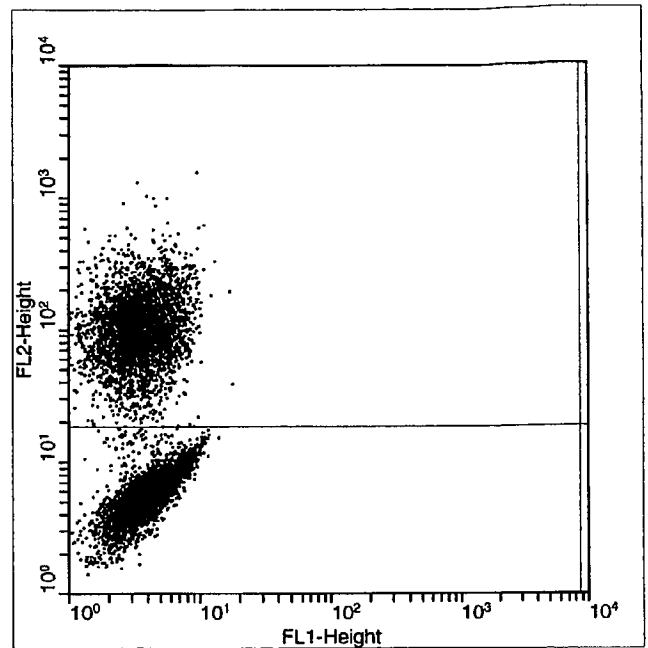
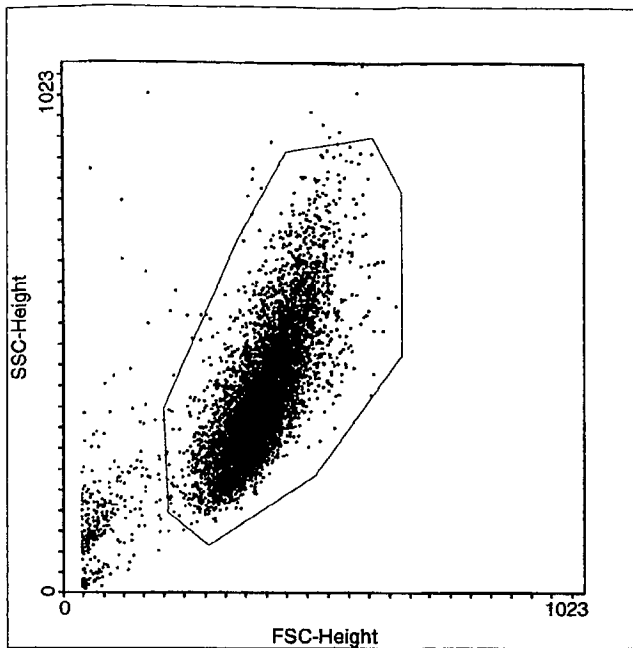
- 0 Gy
- 0 Gy FOR DiI staining (40min)
- 10 Gy
- 20 Gy

ONE SOURCE: 345.59 Rad/min

Ratio: 100μL/100μL

PLATING TIME: 10:00

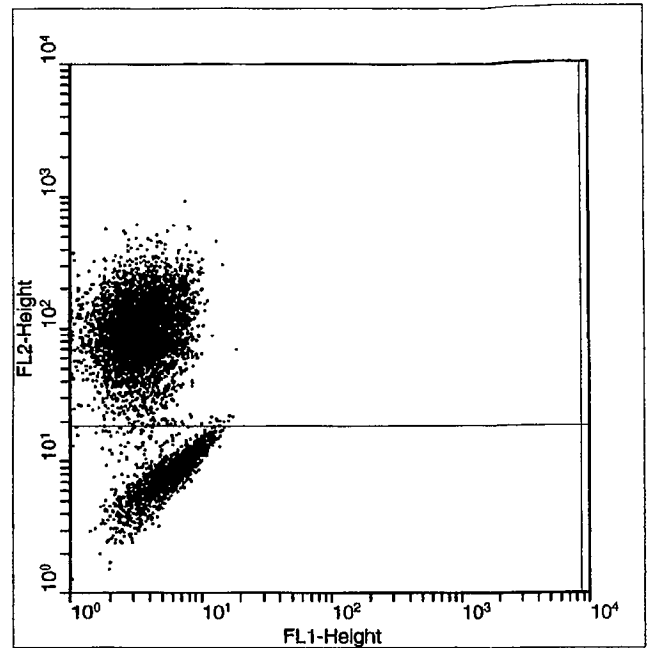
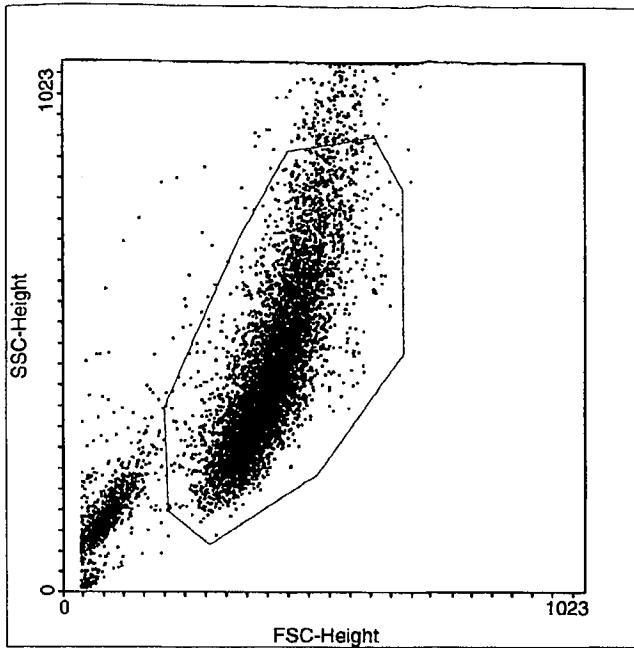




Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:49:11 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061802.013 Sample: 0Gy (DiI) + 0Gy  
 Date: 18-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9566 95.66%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1008,324  

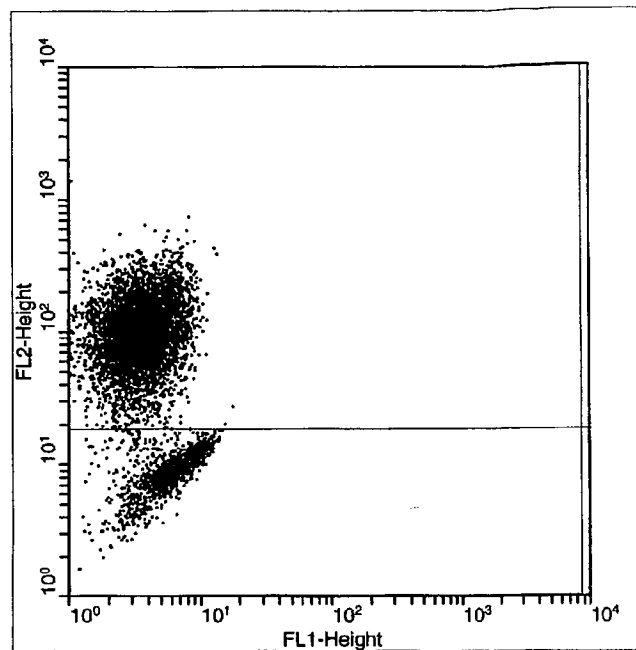
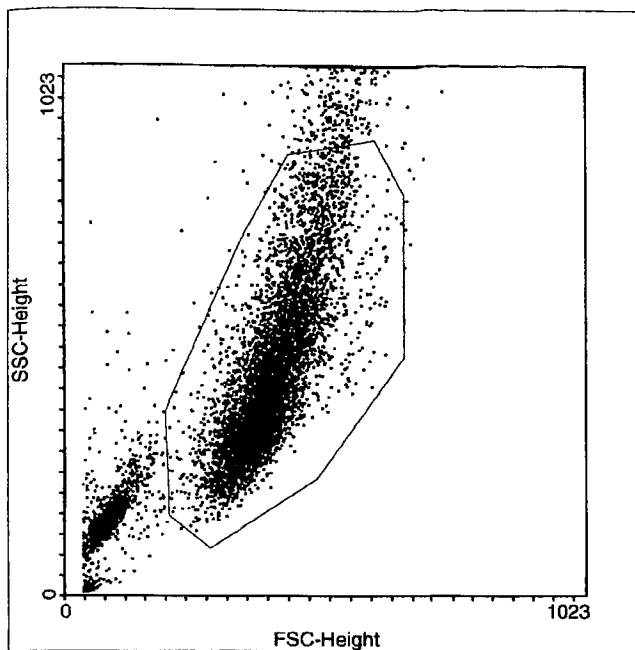
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.5	100.8	4729	47.29	<u>49.44</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.9	5.6	4837	48.37	<u>50.56</u>
4 LR	0.0	0.0	0	0.00	0.00





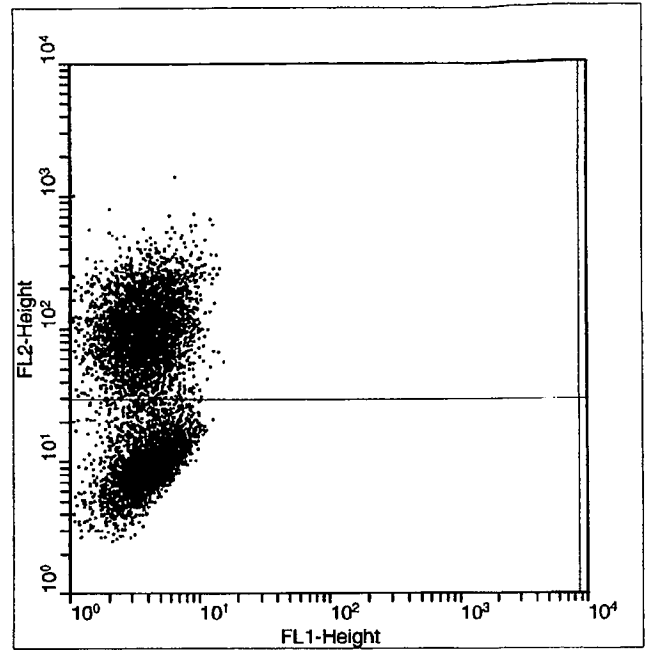
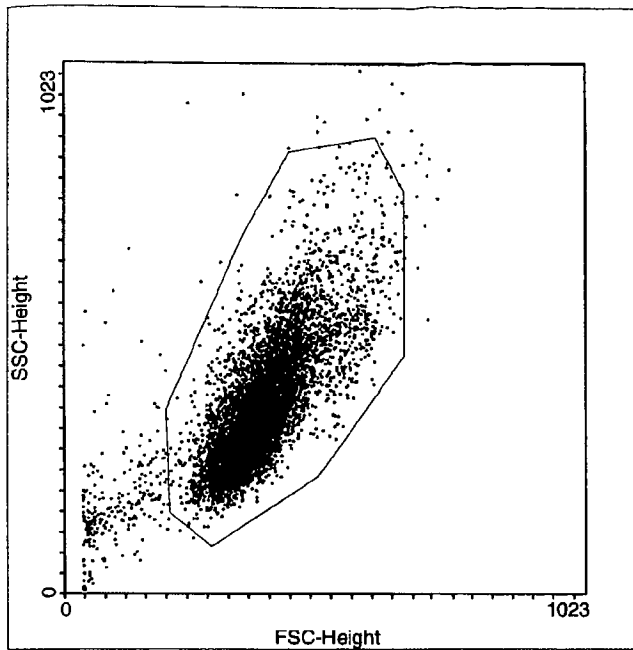
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:49:48 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061802.014 Sample: 0Gy(DiI)+10Gy  
 Date: 18-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8343 83.43%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,324  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.5	96.6	6174	61.74	<u>74.00</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	5.4	7.9	2169	21.69	<u>26.00</u>
4 LR	0.0	0.0	0	0.00	0.00



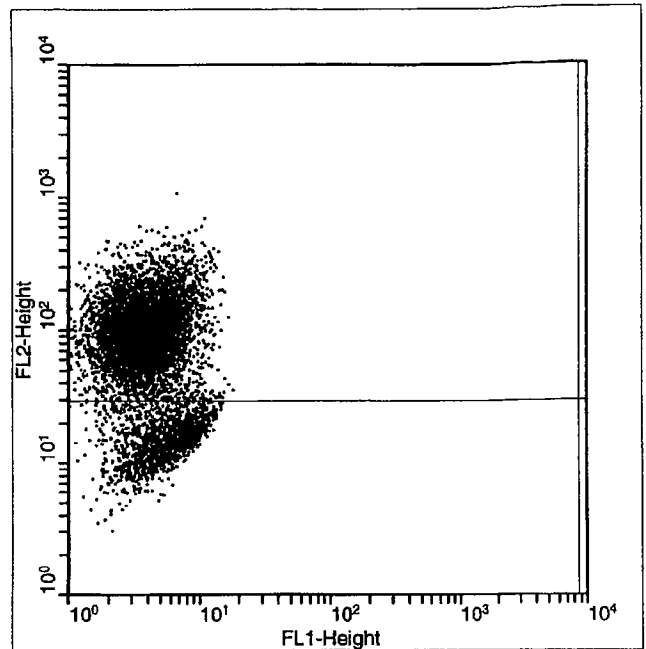
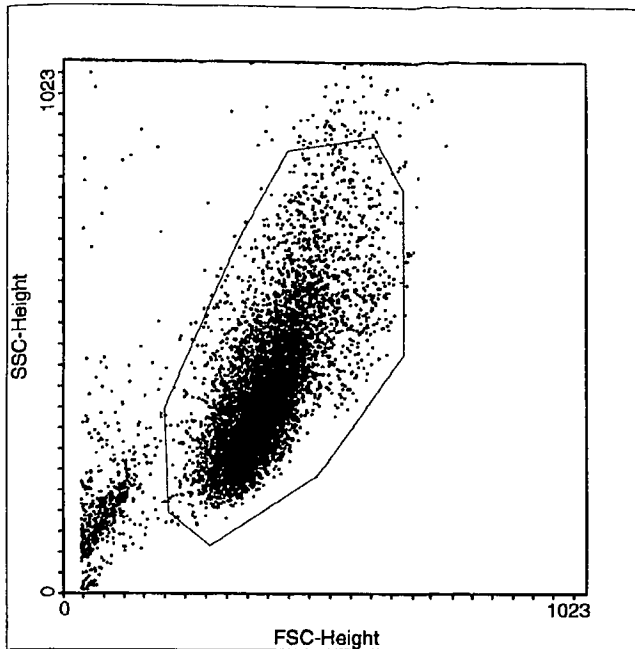
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:50:10 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061802.015 Sample: 0Gy(DiI)+20Gy  
 Date: 18-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 7977 79.77%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,324  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.4	98.4	6432	64.32	80.63
2 UR	0.0	0.0	0	0.00	0.00
3 LL	5.6	8.8	1545	15.45	19.37
4 LR	0.0	0.0	0	0.00	0.00



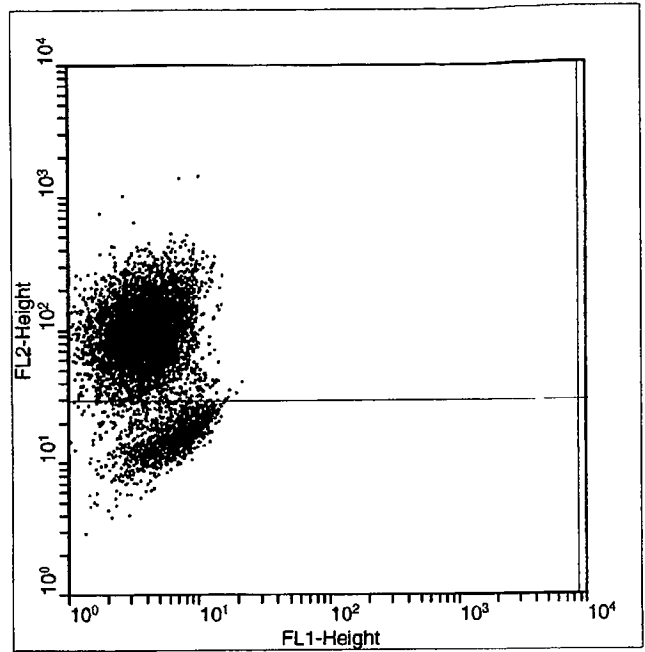
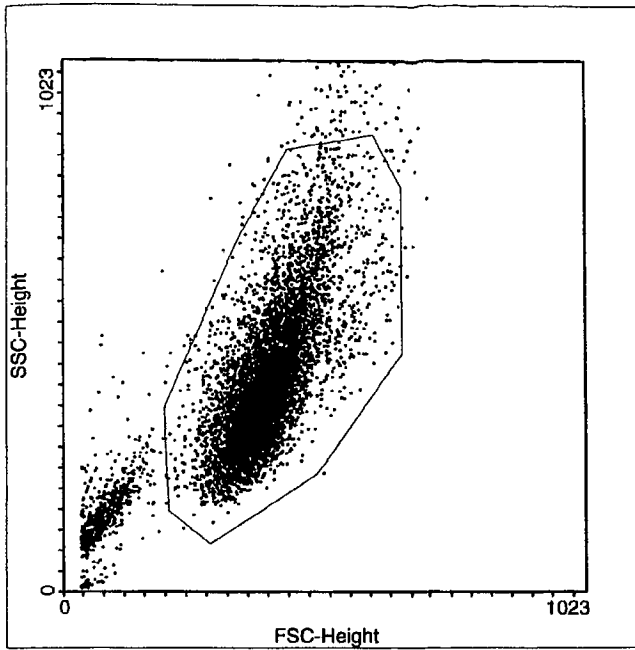
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:51:10 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061802.016 Sample: 0Gy(D11)/0Gy  
 Date: 18-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9637 96.37%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,376  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.8	101.5	5125	51.25	<u>53.18</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.0	9.8	4512	45.12	<u>46.82</u>
4 LR	0.0	0.0	0	0.00	0.00



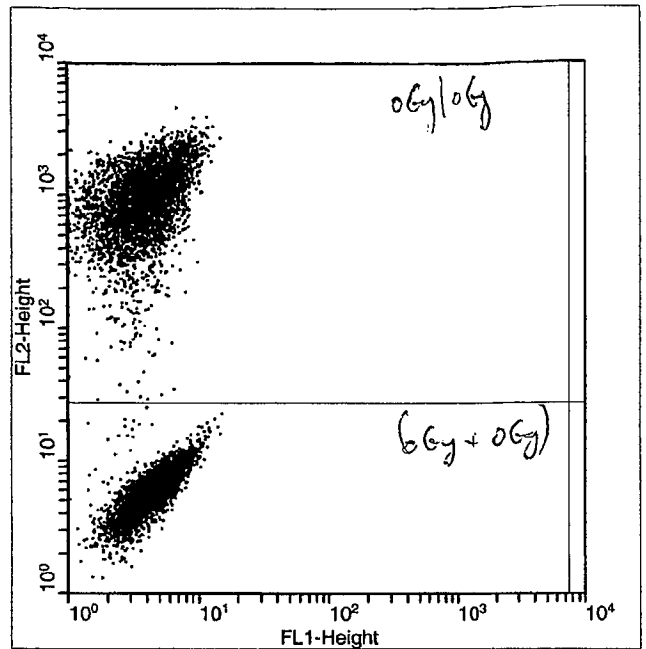
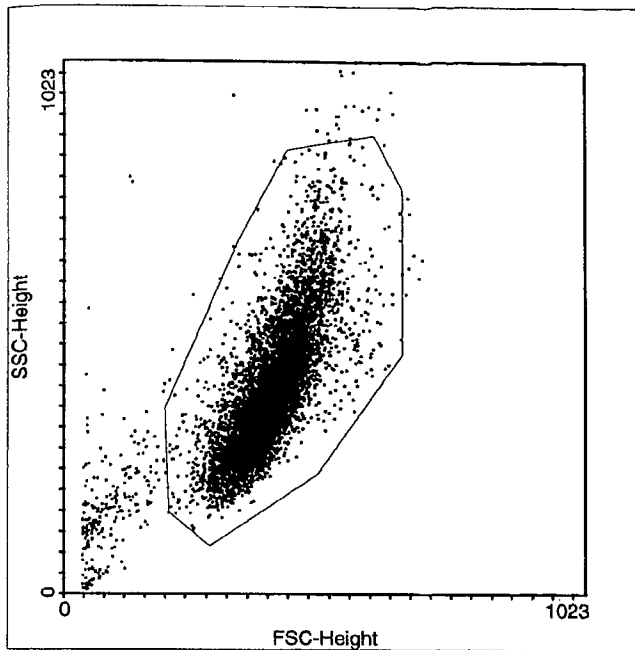
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:52:30 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061802.017 Sample: 0Gy(DiI)/10Gy  
 Date: 18-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8956 89.56%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,376  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.8	100.1	6930	69.30	77.38
2 UR	0.0	0.0	0	0.00	0.00
3 LL	5.3	14.8	2026	20.26	22.62
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:52:50 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061802.018 Sample: (0Gy(DiI)/20Gy)  
 Date: 18-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8914 89.14%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1008,376  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.9	95.8	7150	71.50	80.21
2 UR	0.0	0.0	0	0.00	0.00
3 LL	5.7	15.9	1764	17.64	19.79
4 LR	0.0	0.0	0	0.00	0.00

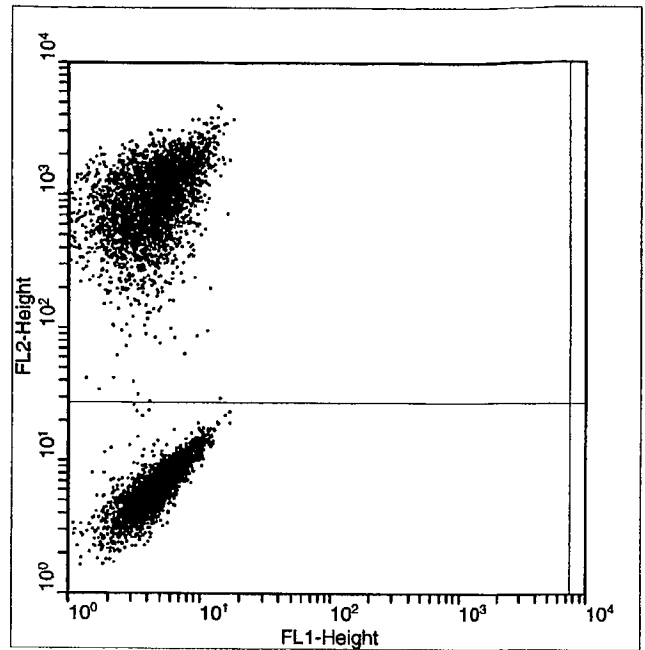
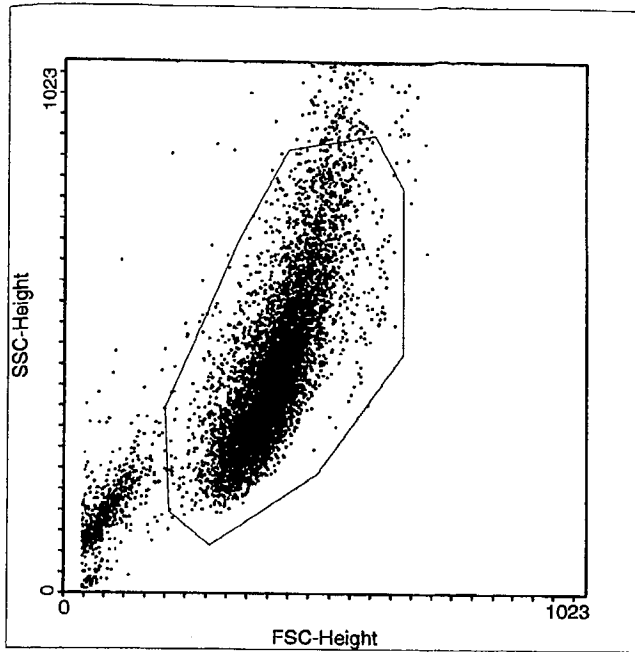


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:43:57 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061802.007 Sample: (0Gy+0Gy)+0Gy/0Gy (DiI)  
 Date: 18-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9648 96.48%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 992,368

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.7	895.3	4941	49.41	51.21
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.4	5.9	4707	47.07	48.79
4 LR	0.0	0.0	0	0.00	0.00

*Handwritten notes:*  
 - 0Gy/0Gy (next to 51.21)  
 - (0Gy+0Gy) (next to 48.79)



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:44:38 2002  
 Gates: R1

Project: Experiment:  
 File: 061802.008 Sample: (0Gy+10Gy)+0Gy/10Gy(DiI)

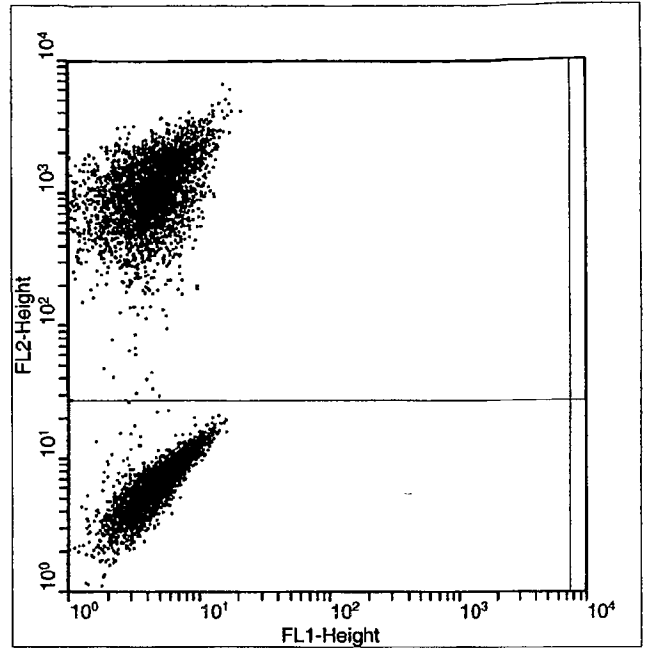
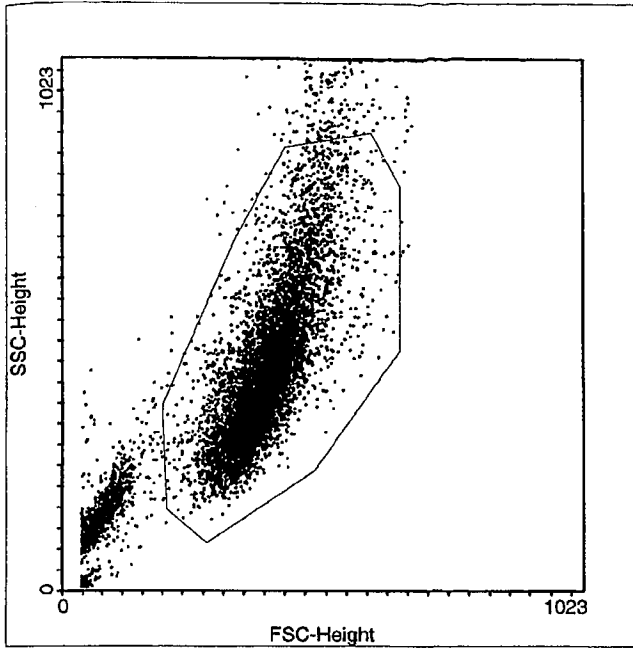
Date: 18-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8800 88.00%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 992,368

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.3	896.9	4811	48.11	54.67
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.7	6.3	3989	39.89	45.33
4 LR	0.0	0.0	0	0.00	0.00

*Handwritten notes:*  
 0Gy/10Gy  
 (0Gy+10Gy)



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:45:14 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061802.009 Sample: (0Gy/20Gy)+0Gy/20Gy (DiT)  
 Date: 18-Jun-2 Parameters:  
 Total Events 10000 Gated Events 8437 84.37%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 992,368  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.3	1013.4	4597	45.97	54.49
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.6	6.2	3840	38.40	45.51
4 LR	0.0	0.0	0	0.00	0.00

!

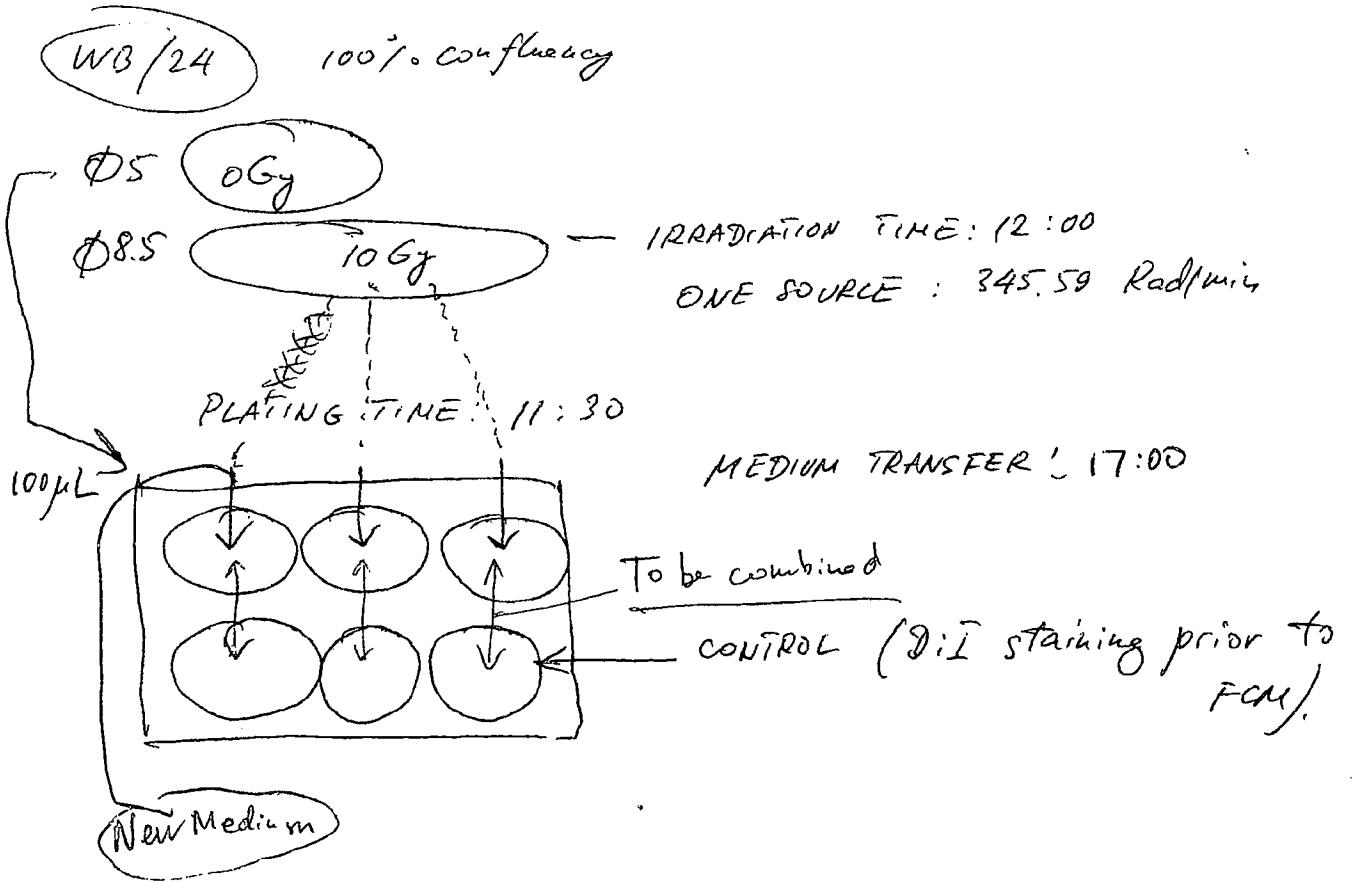


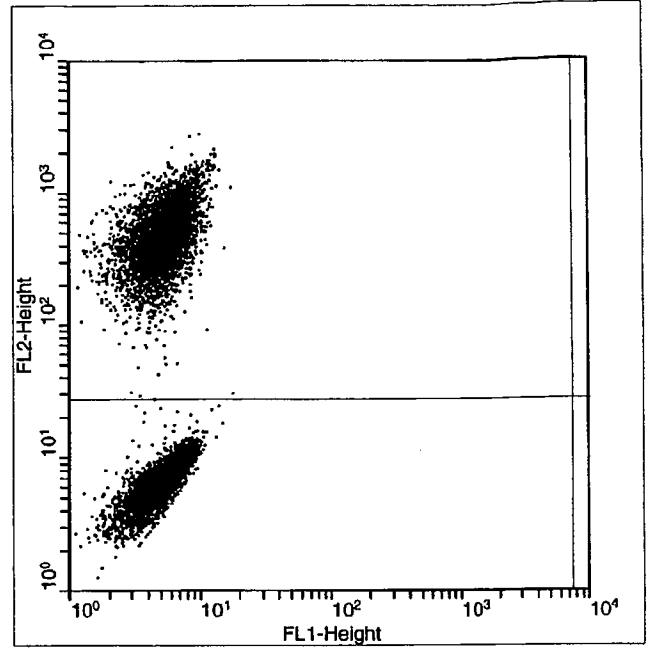
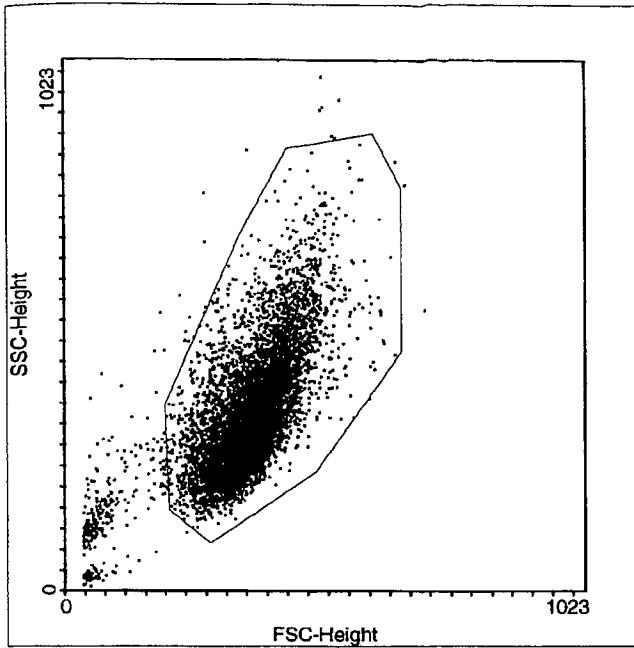
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*Medium  
Transfer*

BYSTANDER EFFECTS ( $\gamma$ -IRRADIATION)  
MEDIUM TRANSFER

JUNE 17, 2002



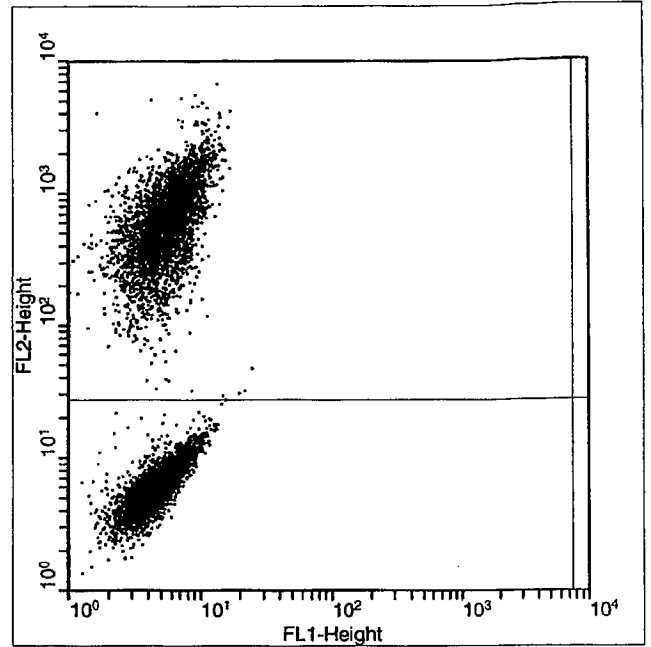
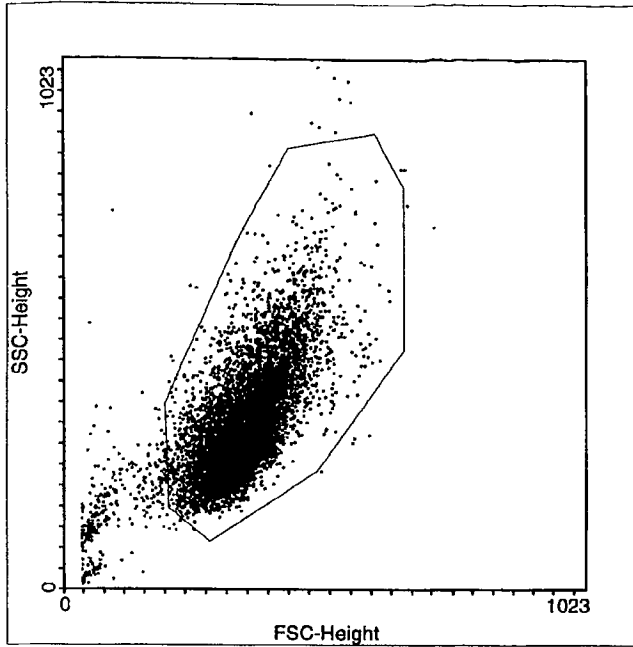


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:45:56 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061802.010 Sample: New Med + Control (DiI)  
 Date: 18-Jun-2 Parameters: /  
 Total Events 10000 Gated Events 5602 96.02%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 992,368

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.2	460.6	5194	51.94	54.09
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.8	6.4	4408	44.08	45.91
4 LR	0.0	0.0	0	0.00	0.00

~~NEW MEDIUM CONTROL~~  
~~CONTROL~~ NEW MEDIUM



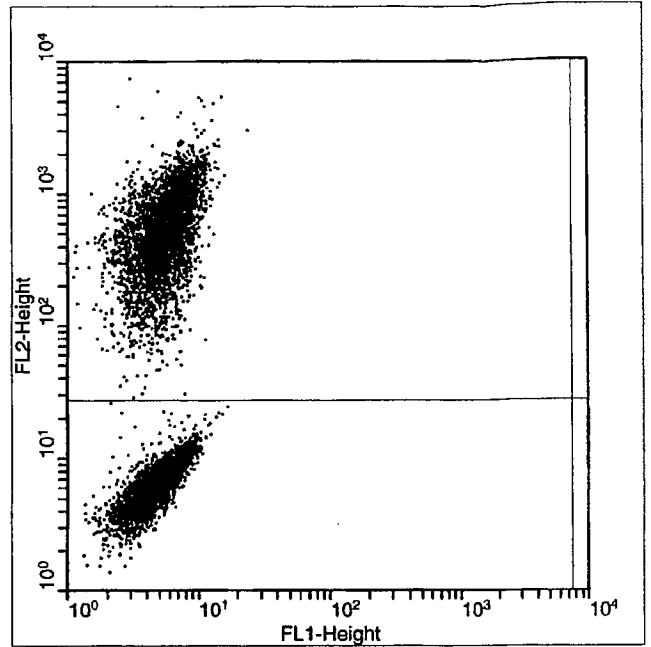
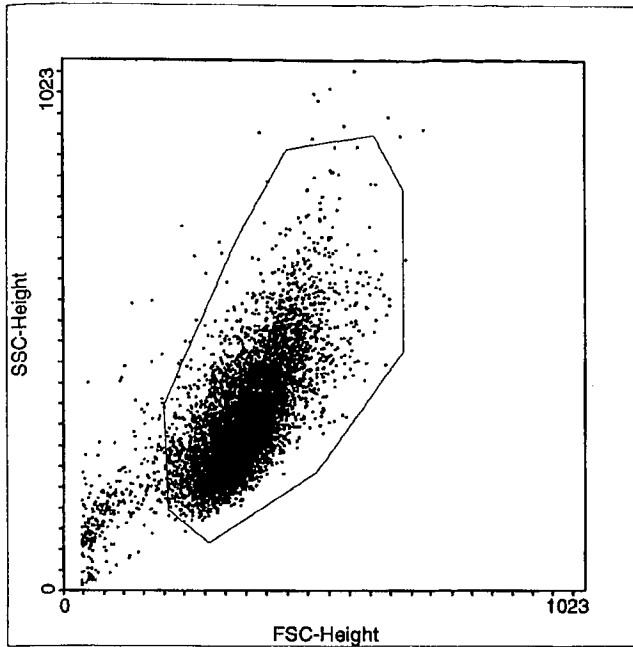
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:47:10 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061802.011 Sample: 10Gy Med + Control (DiT)  
 Date: 18-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9556 95.56%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)

Quadrant x,y: 992,368

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.6	596.4	4810	48.10	50.33
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.8	6.4	4746	47.46	49.67
4 LR	0.0	0.0	0	0.00	0.00

*10 Gy med from CONTROL*  
*control 10 Gy med*



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Wed Jun 19 09:48:22 2002  
 Gates: R1  
 Project: Experiment:  
 File: 061802.012 Sample: 10Gy Med + Control(DiI)  
 Date: 18-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9580 95.80%  
 System: Log Parameter Means: Geometric  
 Quad Stats

FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 992,368

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.4	496.4	4923	49.23	51.39
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.7	6.2	4657	46.57	48.61
4 LR	0.0	0.0	0	0.00	0.00

*control*  
*10 Gy medium*

#6

BYSTANDER EFFECTS (γ-IRRADIATION)

JUNE 20, 2002

WB/25 100% Confluency

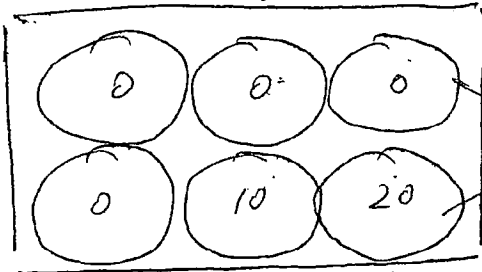
0 Gy  
10 Gy  
20 Gy

IRRADIATION TIME: 9:00

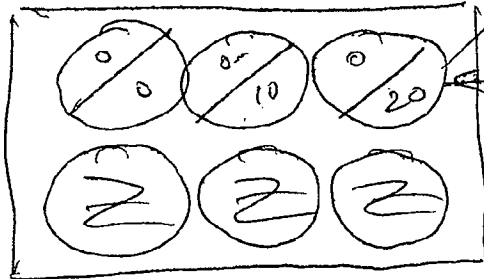
ONE SOURCE: 345.59 Rad/min

PLATING TIME: 10:30

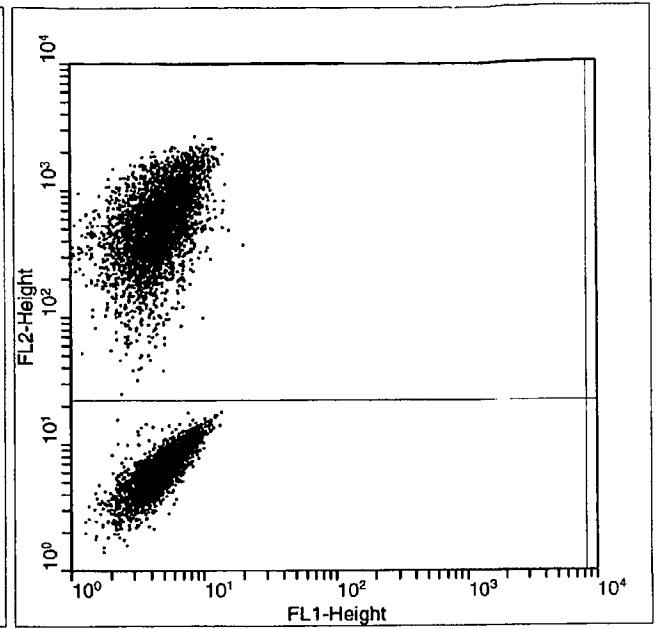
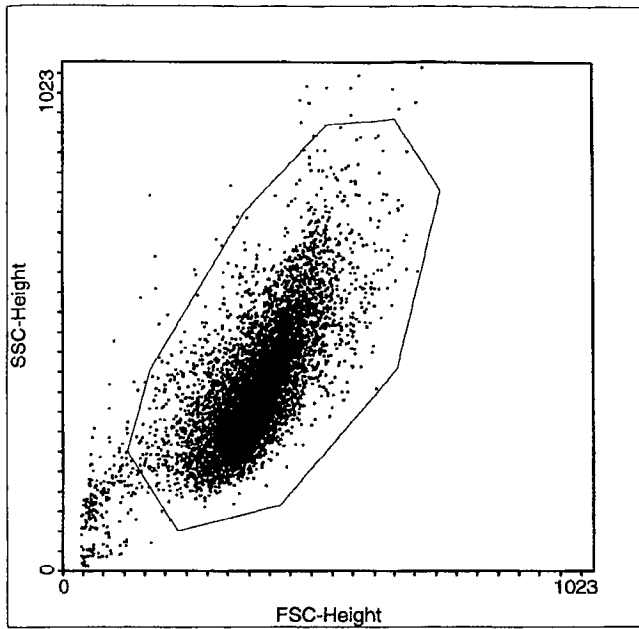
Ratio: 100 μL / 100 μL



To be combined



To be stained prior to FCM  
(15 min)

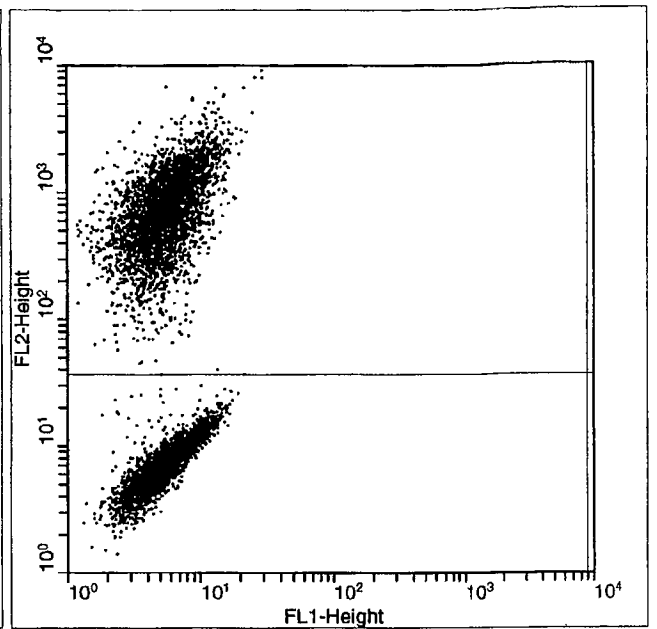
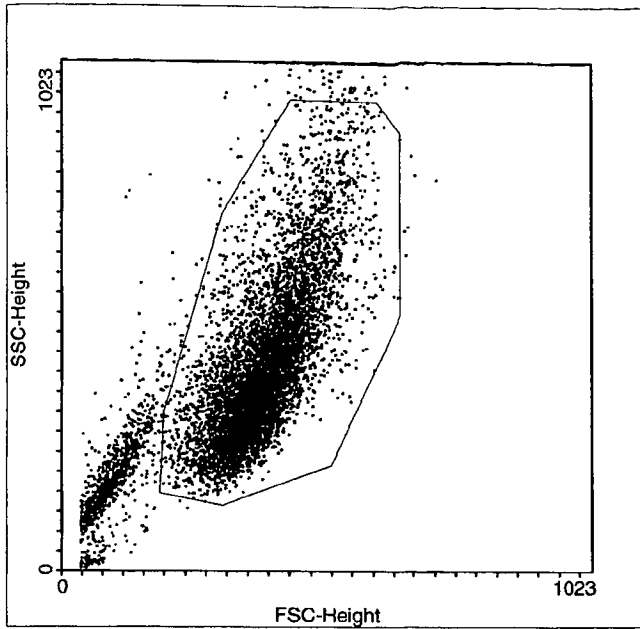


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 21 16:10:43 2002  
 Gates: R1  
 Project: Experiment:  
 File: 062102.001 Sample: (0Gy+0Gy)+(0Gy/0Gy) DiI  
 Date: 21-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9677 96.77%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1004,344

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.6	546.5	5365	53.65	55.44
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.6	6.1	4312	43.12	44.56
4 LR	0.0	0.0	0	0.00	0.00





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 21 16:17:59 2002

Gates: R1

Project: Experiment:

File: 062102.002 Sample: (0Gy+10Gy)+(0Gy/10Gy) Dil

Date: 21-Jun-2 Parameters: 7

Total Events 10000 Gated Events 8530 85.30%

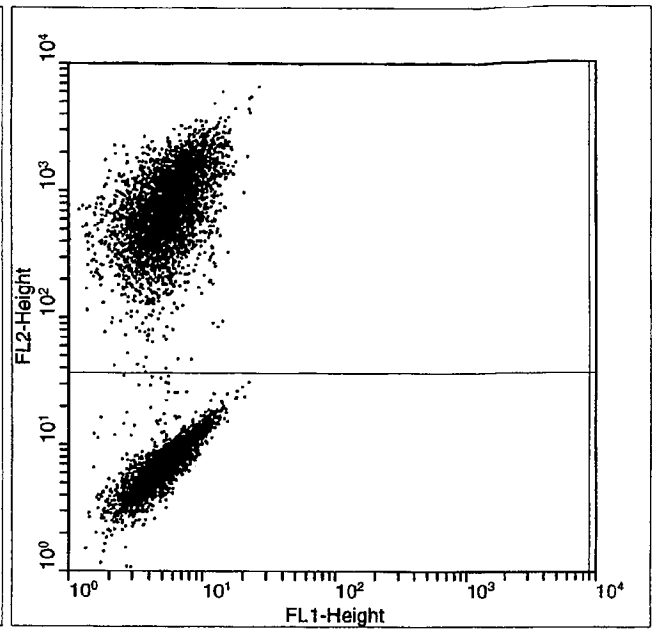
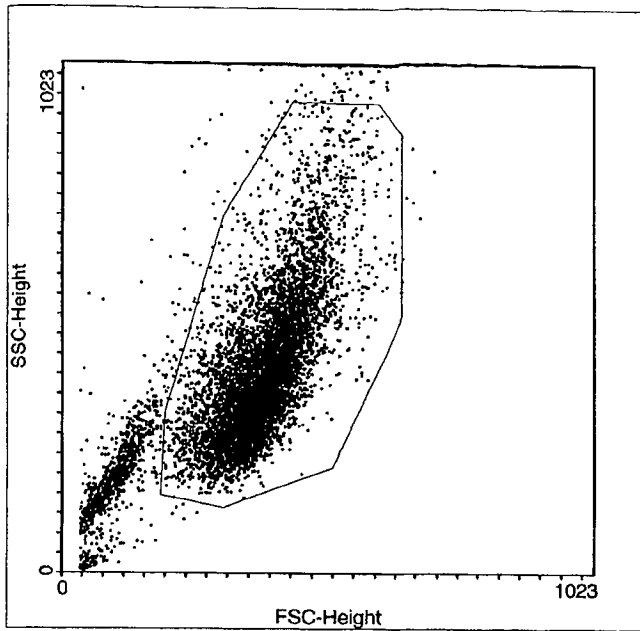
System: Log Parameter Means: Geometric

Quad Stats

FL1-Height (Log) vs FL2-Height (Log)

Quadrant x,y: 1012,400

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.5	700.6	4768	47.68	55.90
2 UR	0.0	0.0	0	0.00	0.00
3 LL	5.3	7.0	3762	37.62	44.10
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 21 16:19:00 2002  
 Gates: R1  
 Project: Experiment:  
 File: 062102.003 Sample: (0Gy+20Gy)+(0Gy/20Gy)Di  
 Date: 21-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 8573 85.73%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1012,400  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.5	710.8	5137	51.37	59.92
2 UR	0.0	0.0	0	0.00	0.00
3 LL	5.1	6.9	3436	34.36	40.08
4 LR	0.0	0.0	0	0.00	0.00

BYSTANDER EFFECTS (f-IRRADIATION)  
MEDIUM TRANSFER

JUNE 20, 200

WB/25 100% confluency

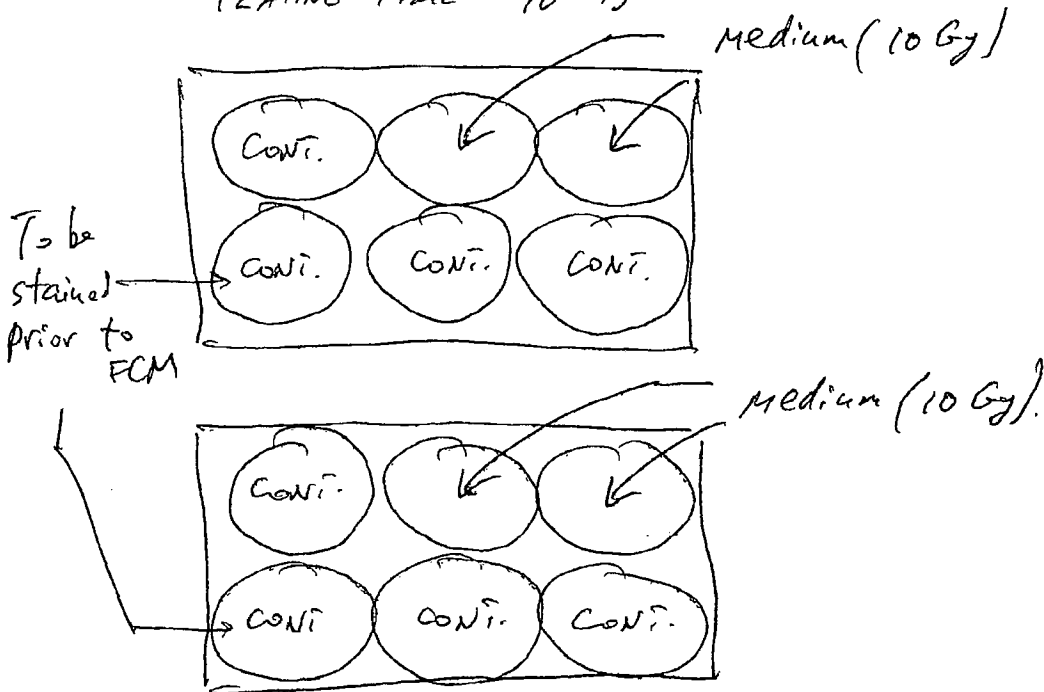
Ø5 0 Gy

Ø8.5 10 Gy

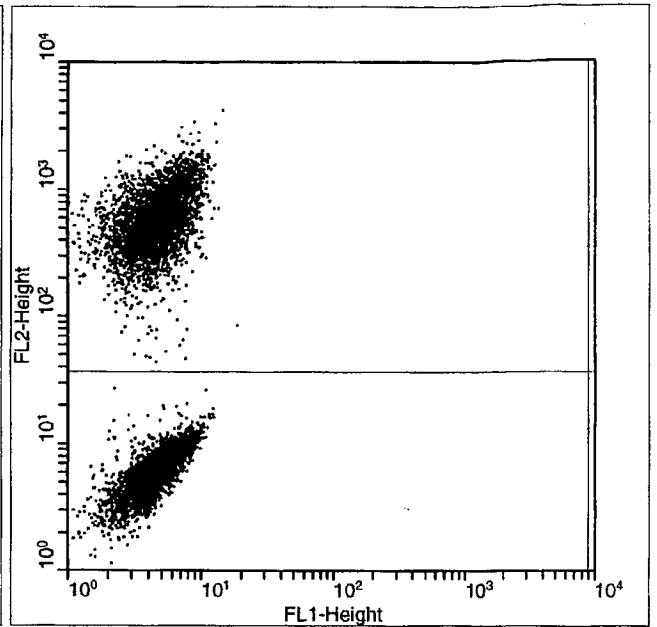
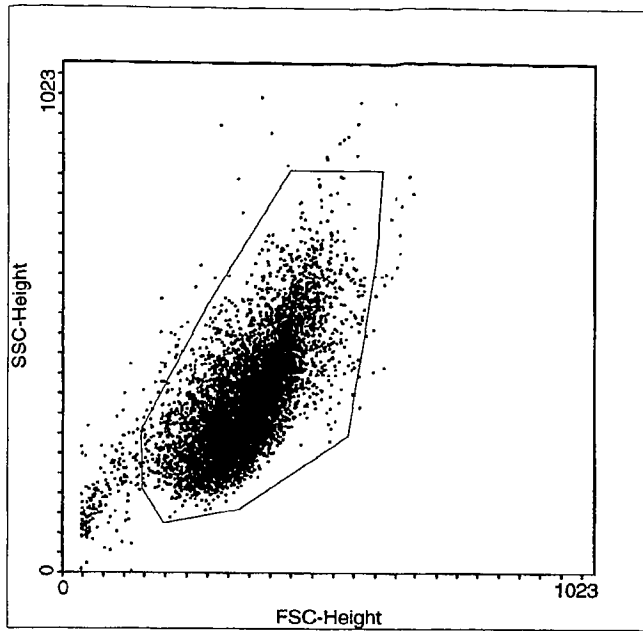
IRRADIATION TIME: 11:00

ONE SOURCE: 345.59 Rad/m<sup>2</sup>

PLATING TIME: 10:45

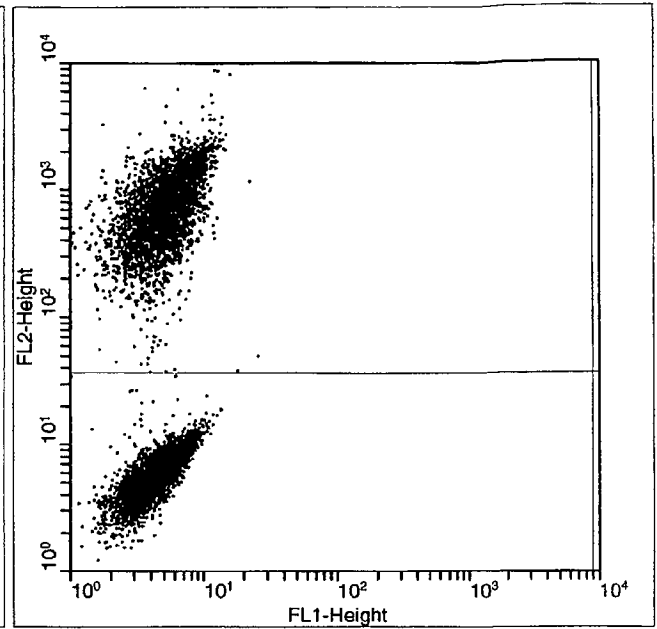
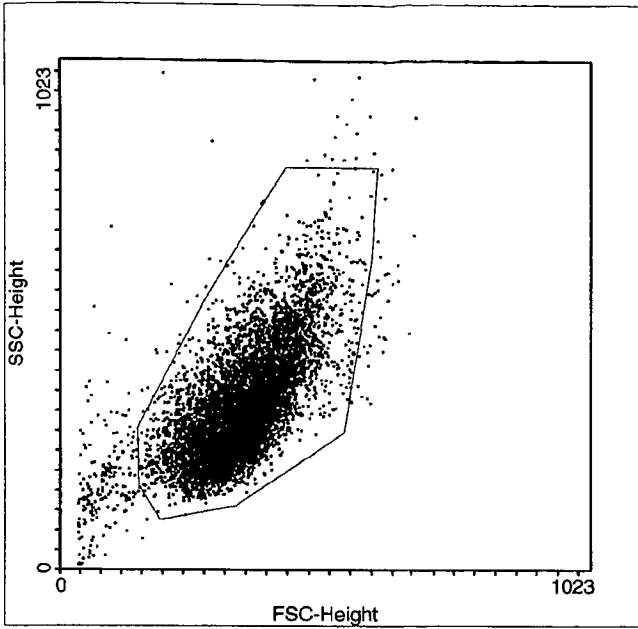


MEDIUM TRANSFER: 17:50



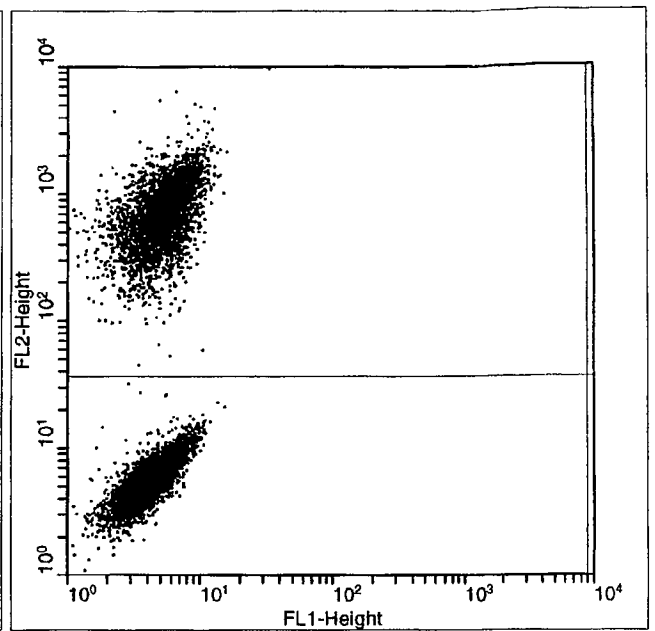
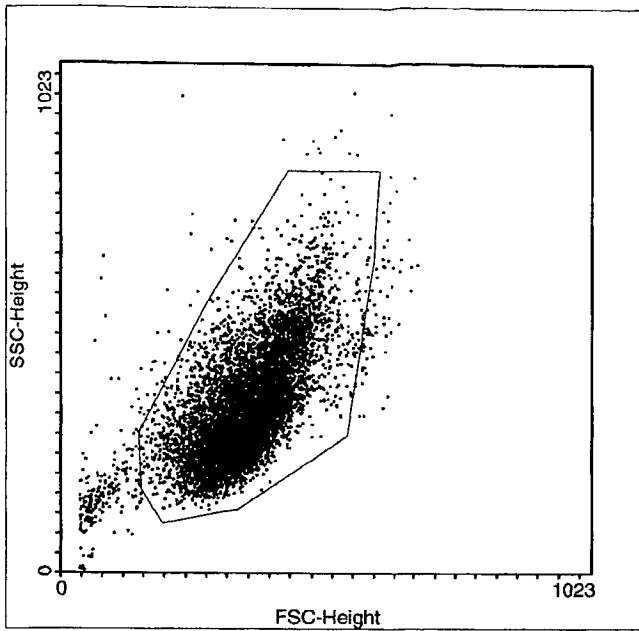
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 21 16:24:09 2002  
 Gates: R1  
 Project: Experiment:  
 File: 062102.004 Sample: Cont.+Cont.(DiI)  
 Date: 21-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9693 96.93%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1012,400  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.7	573.7	5018	50.18	51.77
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.4	5.9	4675	46.75	48.23
4 LR	0.0	0.0	0	0.00	0.00



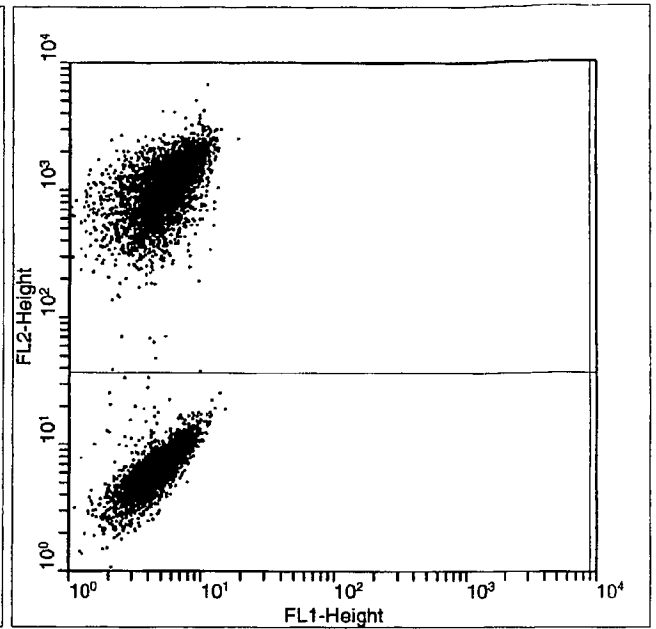
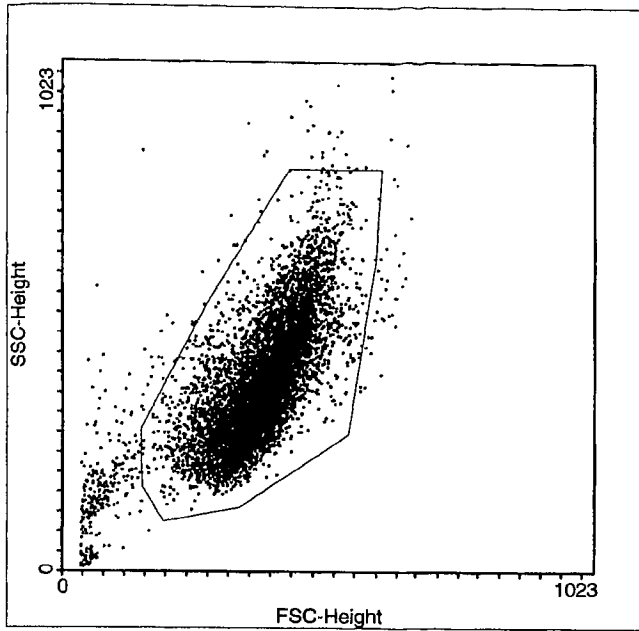
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 21 16:29:21 2002  
 Gates: R1  
 Project: Experiment:  
 File: 062102.006 Sample: 10Gy+Cont. (DiI)  
 Date: 21-Jun-2 Parameters:  
 Total Events 10000 Gated Events 9618 96.18%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1012,400

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.1	684.7	4359	43.59	45.32
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.3	5.7	5259	52.59	54.68
4 LR	0.0	0.0	0	0.00	0.00



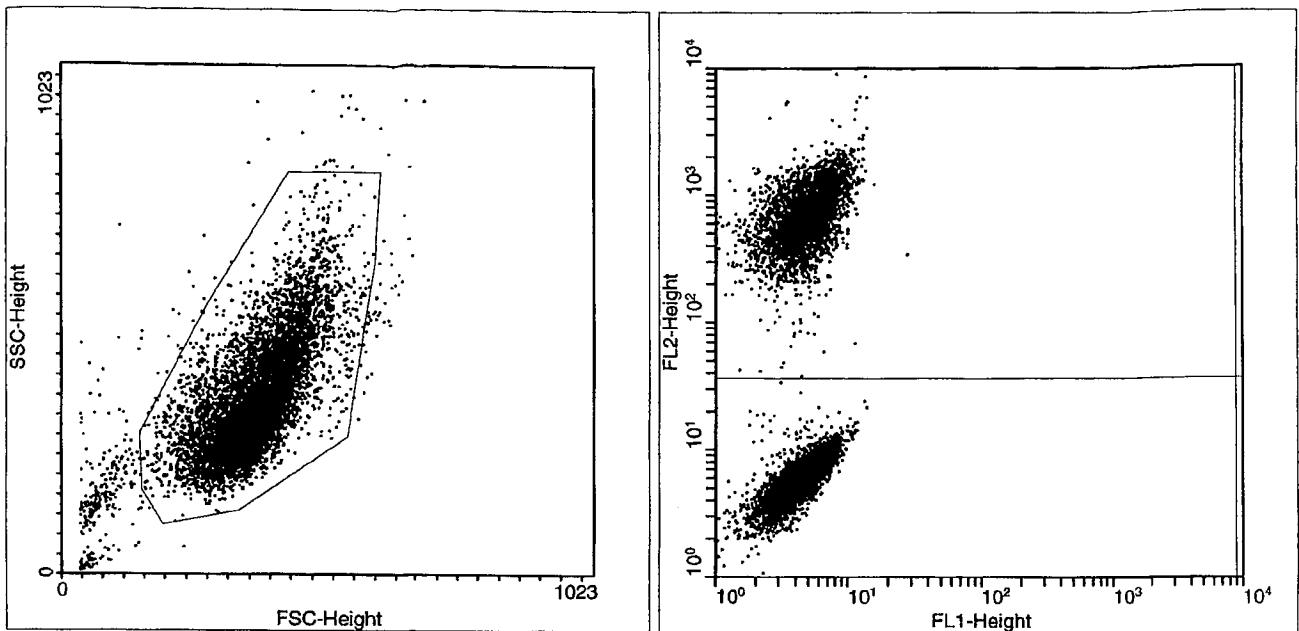
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 21 16:26:36 2002  
 Gates: R1  
 Project: Experiment:  
 File: 062102.005 Sample: 10Gy+Cont. (DiI)  
 Date: 21-Jun-2 Parameters:  
 Total Events 10000 Gated Events 9571 95.71%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x, y: 1012,400  

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.2	678.4	4395	43.95	45.92
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.4	5.8	5176	51.76	54.08
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 21 16:30:36 2002  
 Gates: R1  
 Project: Experiment:  
 File: 062102.007 Sample: (Cont.+Cont.) (DiI)  
 Date: 21-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9541 95.41%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1012,400

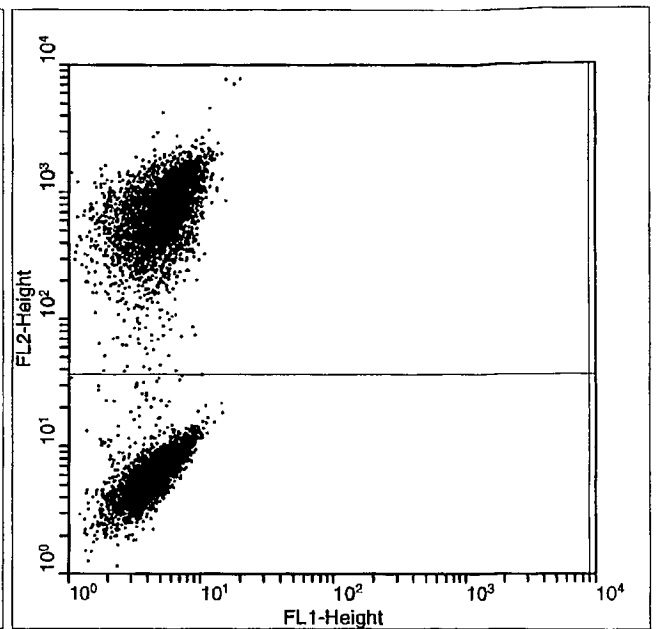
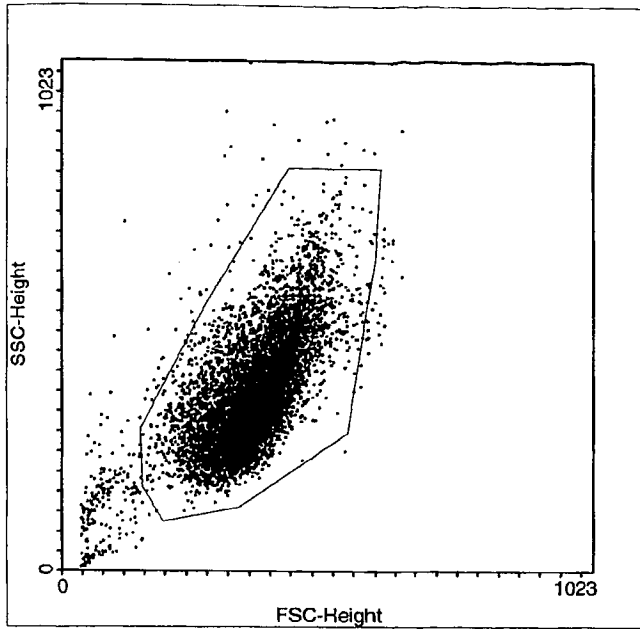
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.2	1003.3	4798	47.98	50.29
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.7	6.3	4743	47.43	49.71
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 21 16:31:50 2002  
 Gates: R1  
 Project: Experiment:  
 File: 062102.008 Sample: 10Gy+Cont. (DiI)  
 Date: 21-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9546 95.46%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FLL-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1012,400

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.9	682.0	4633	46.33	48.53
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.3	5.7	4913	49.13	51.47
4 LR	0.0	0.0	0	0.00	0.00





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Fri Jun 21 16:32:36 2002  
 Gates: R1  
 Project: Experiment:  
 File: 062102.009 Sample: (10Gy+Cont. (DiI))  
 Date: 21-Jun-2 Parameters: 7  
 Total Events 10000 Gated Events 9599 95.99%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1012,400

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	5.0	659.2	4923	49.23	51.29
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.3	5.9	4676	46.76	48.71
4 LR	0.0	0.0	0	0.00	0.00

女子

BYSTANDER EFFECTS ( $\gamma$ -IRRADIATION)

JUNE 28, 2001

WB/25 100% confluency

0Gy

10Gy

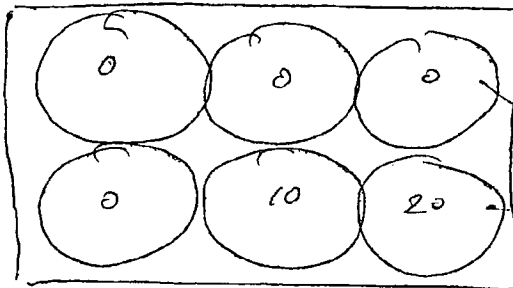
20Gy

IRRADIATION TIME: 9:45

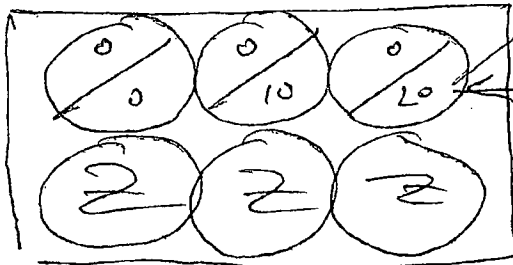
ONE SOURCE: 345.59 Rad/mi

Ratios: 100 $\mu$ l / 100 $\mu$ l.

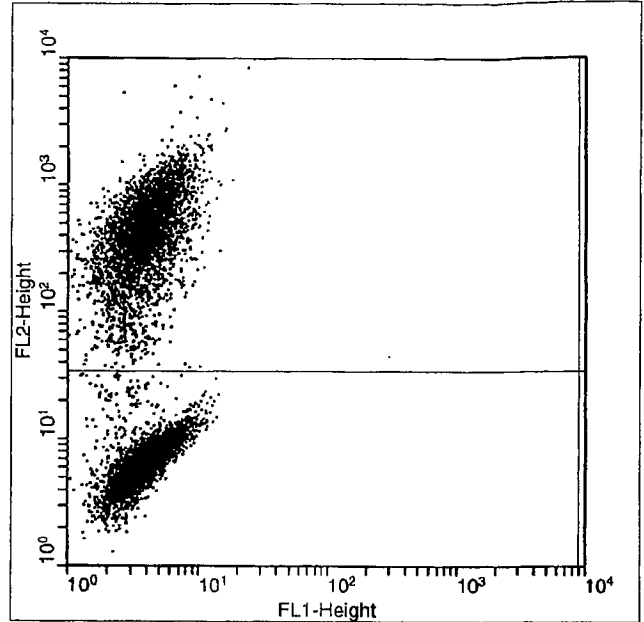
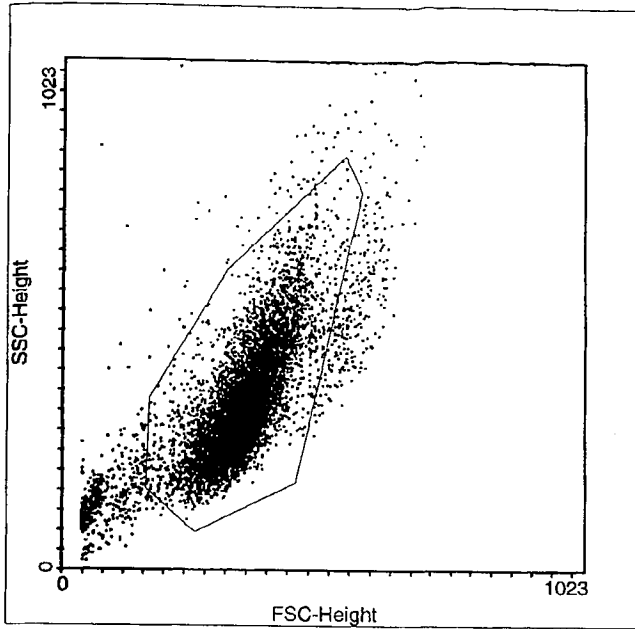
PLATING TIME: 11:00



To be combined

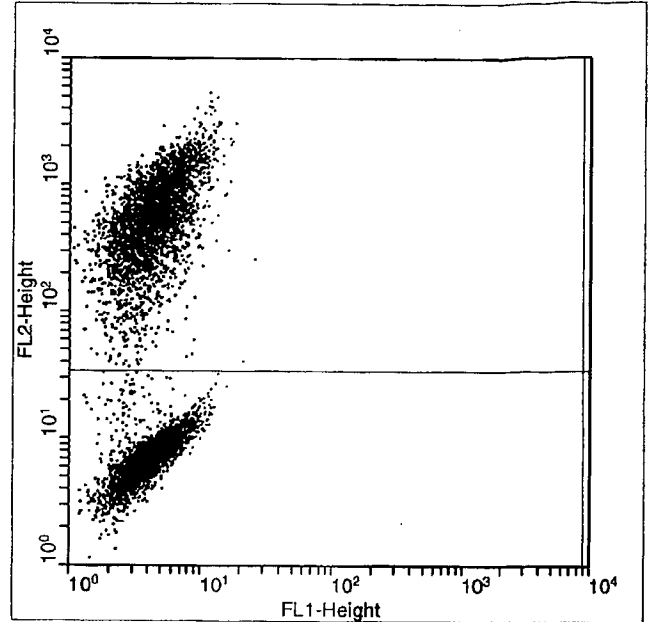
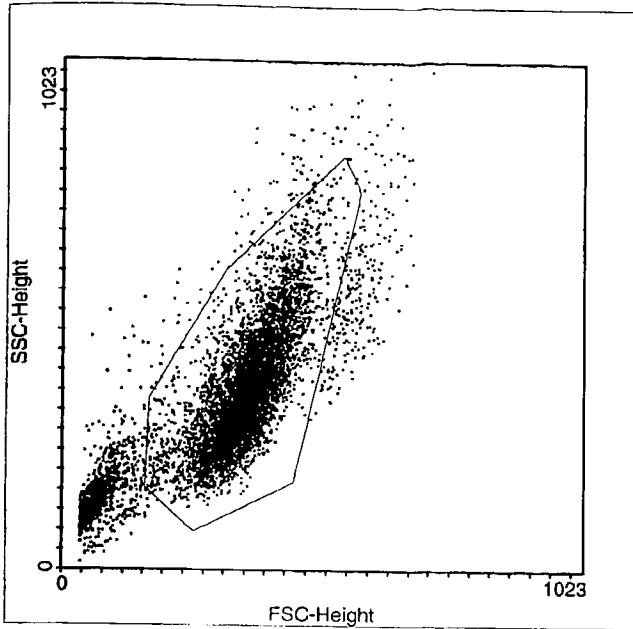


To be stained prior to F1  
(15min)



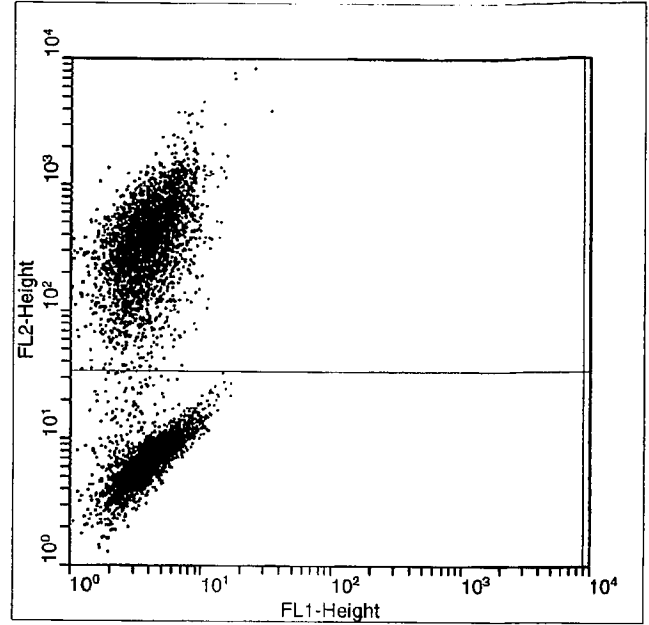
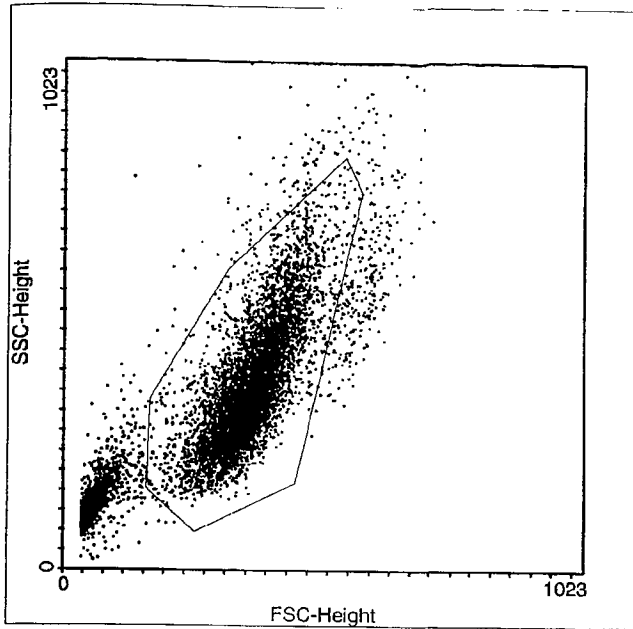
Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jul 01 16:40:11 2002  
 Gates: R1  
 Project: Experiment:  
 File: 070102.001 Sample: ((0Gy+0Gy)/(0Gy/0Gy))\*  
 Date: 1-Jul-2 Parameters:  
 Total Events 10000 Gated Events 8987 89.87%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1012,392

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.9	403.8	4506	45.06	50.14
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.8	6.6	4481	44.81	49.86
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jul 01 16:44:57 2002  
 Gates: R1  
 Project: Experiment:  
 File: 070102.002 Sample: (0Gy+10Gy) / (0Gy/10Gy)\*  
 Date: 1-Jul-2 Parameters: 7  
 Total Events 10000 Gated Events 8015 80.15%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1012,392

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.4	535.8	4347	43.47	54.24
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.1	6.9	3668	36.68	45.76
4 LR	0.0	0.0	0	0.00	0.00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jul 01 16:46:22 2002  
 Gates: R1

Project: Experiment:  
 File: 070102.003 Sample: ((0Gy+20Gy)/(0Gy/20Gy))\*  
 Date: 1-Jul-2 Parameters:

Total Events 10000 Gated Events 7916 79.16%  
 System: Log Parameter Means: Geometric

Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1012,392

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	3.9	353.7	4301	43.01	54.33
2 UR	0.0	0.0	0	0.00	0.00
3 LL	4.0	6.9	3615	36.15	45.67
4 LR	0.0	0.0	0	0.00	0.00

BYSTANDER EFFECTS (f-IRRADIATION)  
MEDIUM TRANSFER

JUNE 28, 2002

WB / 25 100% Confluency

Ø 5

0 Gy

Ø 8.5

10 Gy

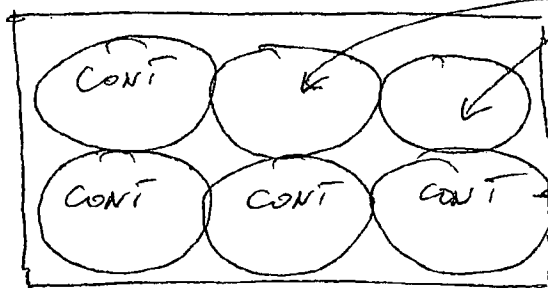
IRRADIATION TIME: 12:00

ONE SOURCE: 345.59 Rad/m<sup>2</sup>

Plating time: 11:40

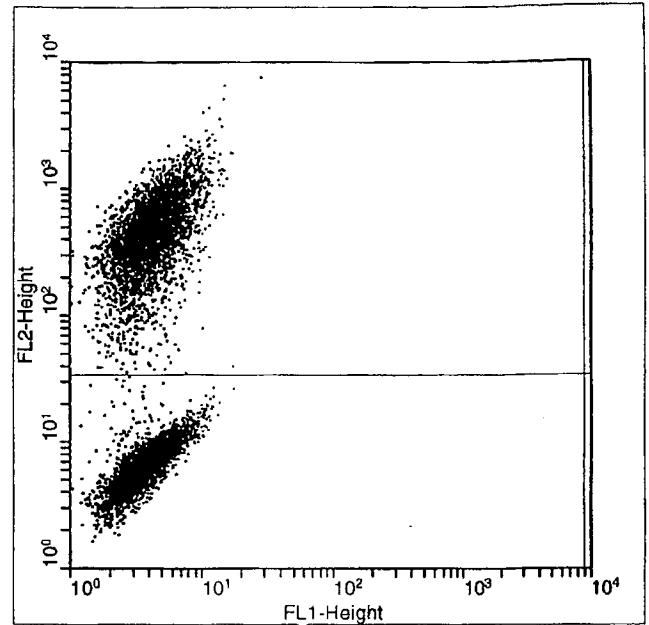
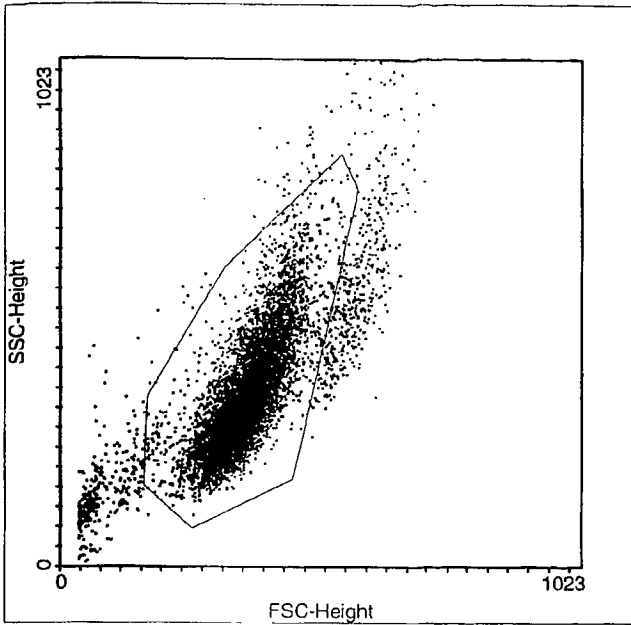
100µL

MEDIUM (10 Gy)



To be stained prior to  
ECM

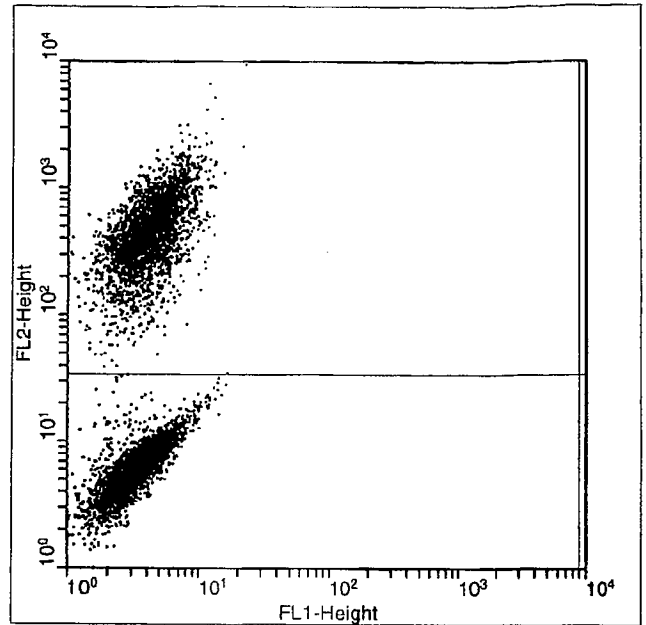
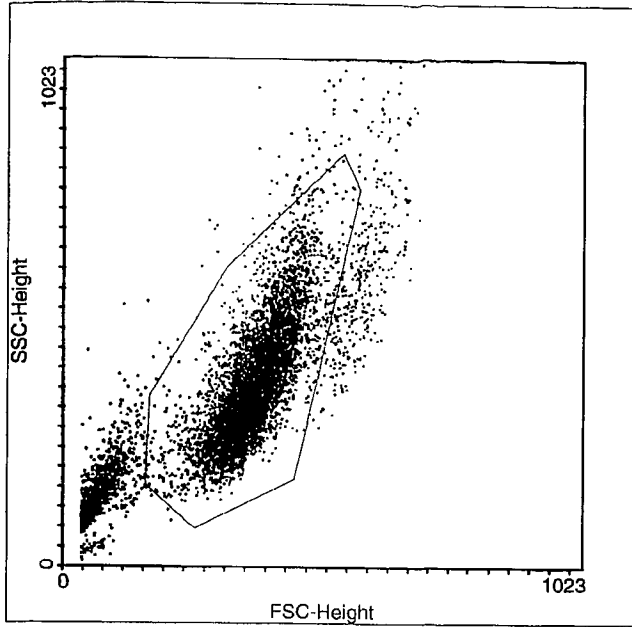
MEDIUM TRANSFER: 17:00



Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jul 01 16:46:55 2002  
 Gates: R1  
 Project: Experiment:  
 File: 070102.004 Sample: 0Gy+0Gy\*  
 Date: 1-Jul-2 Parameters: 7  
 Total Events 10000 Gated Events 8868 88.68%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1012,392

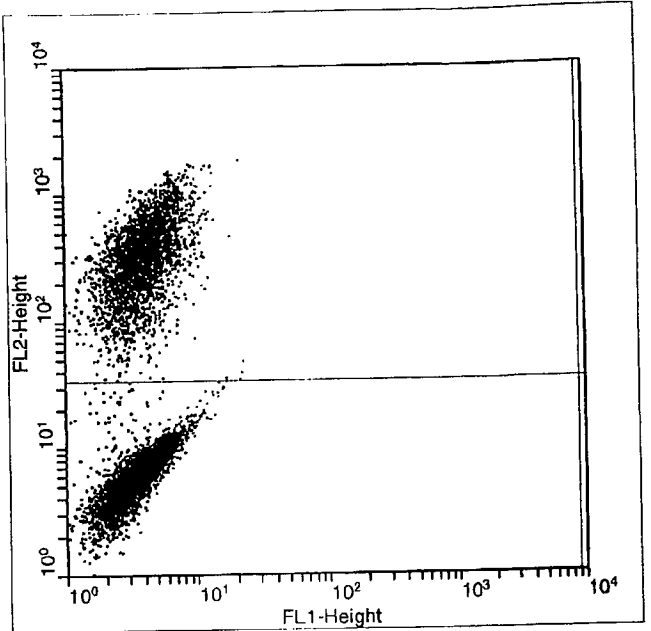
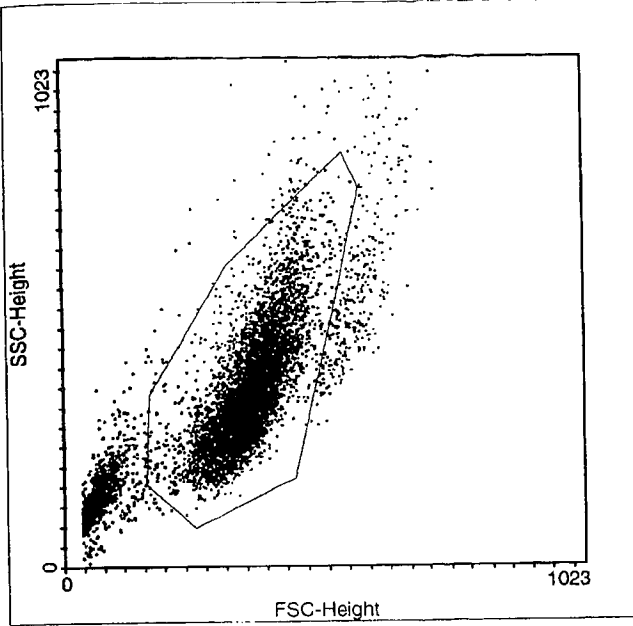
Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.3	435.2	4520	45.20	50.97
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.8	6.7	4348	43.48	49.03
4 LR	0.0	0.0	0	0.00	0.00





Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jul 01 16:48:59 2002  
 Gates: R1  
 Project: Experiment:  
 File: 070102.005 Sample: QCy+10Gy\* (Medium)  
 Date: 1-Jul-2 Parameters: 7  
 Total Events 10000 Gated Events 7882 78.82%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height (Log) vs FL2-Height (Log)  
 Quadrant x,y: 1012,392

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.2	439.4	3592	35.92	<u>45.57</u>
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.3	6.0	4290	42.90	<u>54.43</u>
4 LR	0.0	0.0	0	0.00	0.00

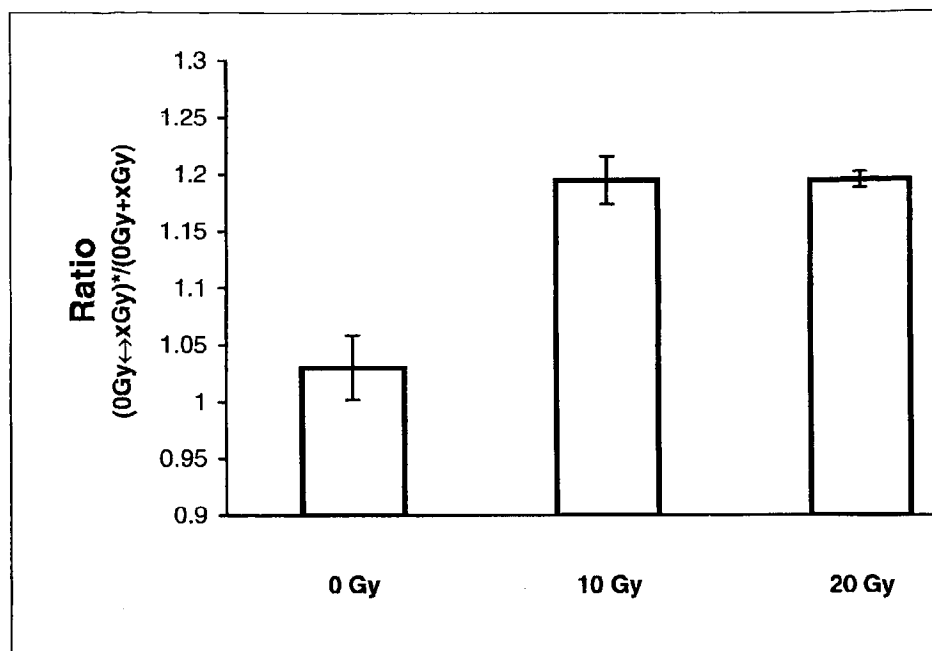


Multiple Document Interface for Flow Cytometry  
 WinMDI Version 2.8 - Windows 3.95/DOS 7.10  
 Mon Jul 01 16:49:25 2002  
 Gates: R1  
 Project: Experiment:  
 File: 070102.006 Sample: (0Gy+10Gy\* (Medium))  
 Date: 1-Jul-2 Parameters: 7  
 Total Events 10000 Gated Events 8047 80.47%  
 System: Log Parameter Means: Geometric  
 Quad Stats  
 FL1-Height(Log) vs FL2-Height(Log)  
 Quadrant x,y: 1012,392

Quad	X-Mean	Y-Mean	Events	%Total	%Gated
1 UL	4.0	309.5	3472	34.72	43.15
2 UR	0.0	0.0	0	0.00	0.00
3 LL	3.4	5.9	4575	45.75	56.85
4 LR	0.0	0.0	0	0.00	0.00

Scheme	Exp. 5	Exp. 7	Avg	SD
$(0\text{Gy} \leftrightarrow x\text{Gy})^*/(0\text{Gy}+x\text{Gy})$	1.05	1.01	1.03	0.028284
$(0\text{Gy} \leftrightarrow 10\text{Gy})^*/(0\text{Gy}+10\text{Gy})$	1.21	1.18	1.195	0.021213
$(0\text{Gy}/20\text{Gy})^*/(0\text{Gy}+20\text{Gy})$	1.2	1.19	1.195	0.007071

0 Gy	1.03
10 Gy	1.195
20 Gy	1.195



Please estimate proliferation state of unirradiated cells in the co-culture system (0Gy↔5Gy), when the number of unirradiated cells (0Gy) is 70%, and the number of irradiated cells (5Gy) is 30%. Ratio of the total cell numbers in culture of unirradiated cell (0Gy↔0Gy) to the total cell number in co-culture system (0Gy↔5Gy) is 1.32.

↑  
57%

↑  
43%

$$R_0 = \frac{f_U(D) P'_I(D)}{f_U(0) + \cancel{P'_I(D)}} \quad \frac{f_U'(D)}$$

$$= \frac{0.7}{0.5} \quad \frac{0.43}{0.57} = 1.056$$

Please estimate proliferation state of unirradiated cells in the co-culture system (0Gy↔5Gy), when the number of unirradiated cells (0Gy) is 70%, and the number of irradiated cells (5Gy) is 30%. Ratio of the total cell numbers in culture of unirradiated cell (0Gy↔0Gy) to the total cell number in co-culture system (0Gy↔5Gy) is 1.325,

↑  
57%

~~57%~~ | ~~43%~~  
43%

$$R_u = \frac{70}{50} \times \frac{1}{1.325} = 1.057.$$