FCS 3.1 – Examples of data transformations: from channel values to computer display

Josef Spidlen, Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Example 1: Integer data type

Dataset header:

```
$MODE/L/     $DATATYPE/I/        $TOT/3/      $PAR/4/
$P1N/FSC-H/ $P1E/0,0/            $P1R/1024/   $P1B/16/ $P1D/Linear,-100,900/
$P2N/SSC-H/ $P2E/0,0/ $P2G/2/ $P2R/1024/      $P2B/16/ $P2D/Linear,100,350/
$P3N/FL1-H/ $P3E/4,1/            $P3R/1024/   $P3B/16/ $P3D/Logarithmic,2,1/
$P4N/FL2-H/ $P4E/4,0.1/          $P4R/1024/   $P4B/16/ $P4D/Logarithmic,3,0.5/

$SPILLOVER/2,FL1-H,FL2-H,1,0.12,0.26,1/

$P3CALIBRATION/1.234,MESF/    $P4CALIBRATION/3.456,MESF/
```

Channel values in file:

```
Event       FSC-H       SSC-H       FL1-H       FL2-H
1           500         460         150         300
2           100         620         300         600
3           800         280         500         900
min         0           0           0           0
max         1023        1023        1023        1023
```

- Minimum value allowed in the file: 0   = 0 always with $DATATYPE/I/
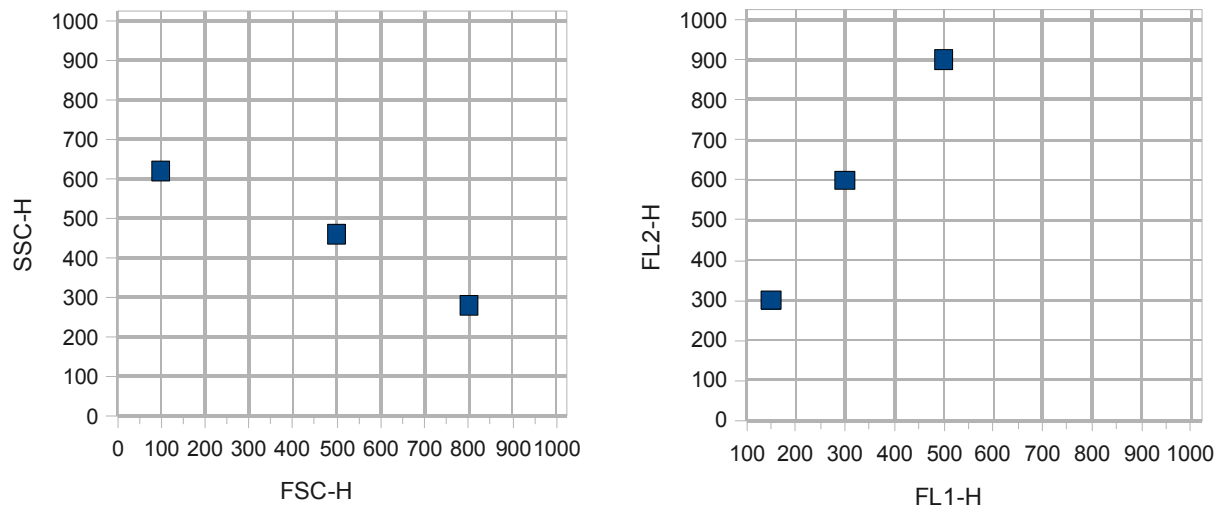- Maximum value allowed in the file: 1023   = $PnR - 1; may vary among different parameters

Figure 1: Example 1 - channel values as in the FCS data file.

Scale values:

```
Event       FSC-H       SSC-H       FL1-H       FL2-H
1           500         230         3.85        1.49
2           100         310         14.86       22.07
3           800         140         89.77       327.81
min         0           0           1           0.1
max         1023        511.5       9910.5      991.05
```

- FSC-H: scale values equal channel values since $P1E/0,0/$ and $P1G$ is not present
- SSC-H: scale values equal channel values divided by the value of the $P2G$ keyword
- FL1-H, FL2-H: For $PnR/r/$, r>0, $PnE/f_1,f_2/$, f_1>0, f_2>0: n is a logarithmic parameter with scale values reaching from f_2 to $10^{\wedge}(f_1+\log(f_2))$. A channel value *xc* can be converted to a scale value *xs* as $xs = 10^{\wedge}(f_1*xc /r) * f_2$. If f_2 equals 0, consider it 1.
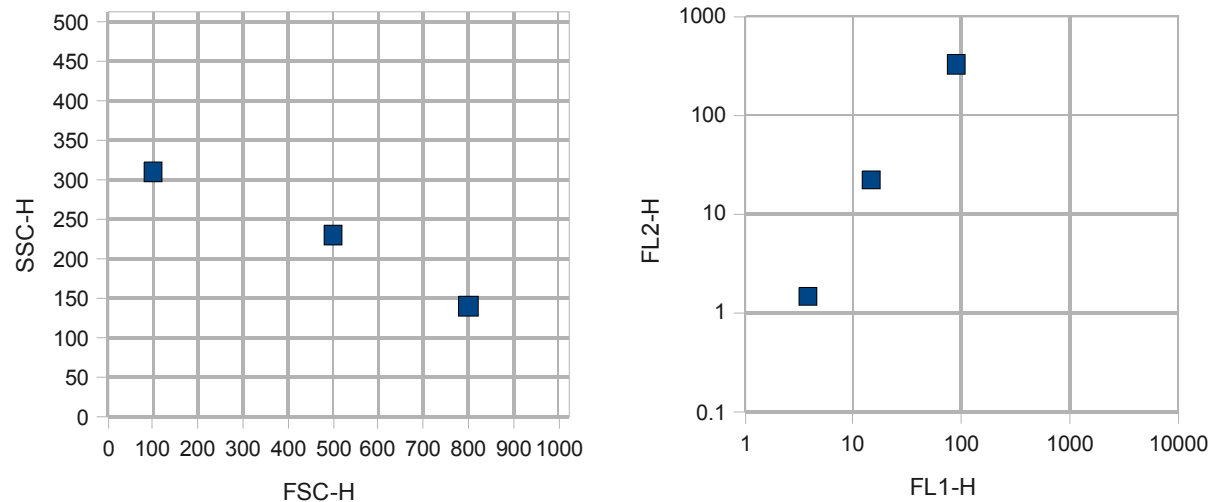
Figure 2: Example 1 - scale values.

Displayed values (as suggested by $PnD):

Event	FSC-H	SSC-H	FL1-H	FL2-H
1	500	230	3.85	1.49
2	100	310	14.86	22.07
3	800	140	89.77	327.81
Displayed min	-100	100	1	0.5
Displayed max	900	350	100	500

- The value of $PnD does not effect the actual values, it only recommends visualization.
- In this case, we have already guessed to use linear visualization for FSC-H and SSC-H and logarithmic visualization for FL1-H and FL2-H. The value of the $PnD keywords allows us to
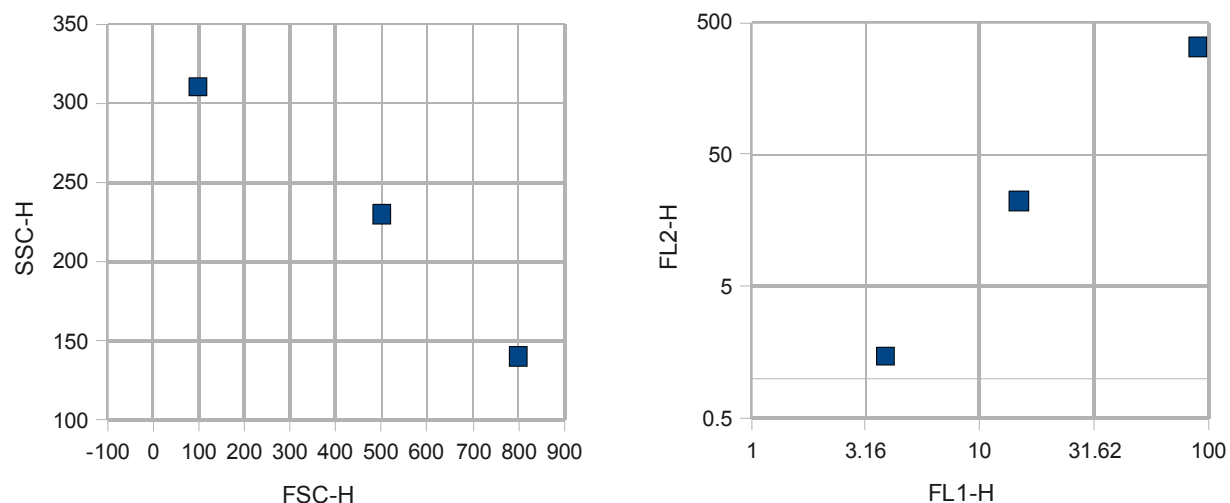- adjust the scale slightly better for our data set.

Figure 3: Example 1 - scale values displayed as suggested by the $PnD keyword.

Compensated values:

Spillover matrix S	FL1-H	FL2-H
FL1-H	1.0	0.12
FL2-H	0.26	1.0

```
Event          FSC-H          SSC-H          FL1-H          FL2-H
1              500            230            3.58           1.06
2              100            310            9.41           20.94
3              800            140            4.68           327.25
```

- The FSC-H and SSC-H parameters are not included in the spillover matrix, thus the values of FSC-H and SSC-H are not affected by the compensation.
- The row vector of compensated FL1-H,FL2-H values can be obtained as the result of matrix multiplication of the row vector of uncompensated FL1-H,FL2-H values by the inverse of the spillover matrix.
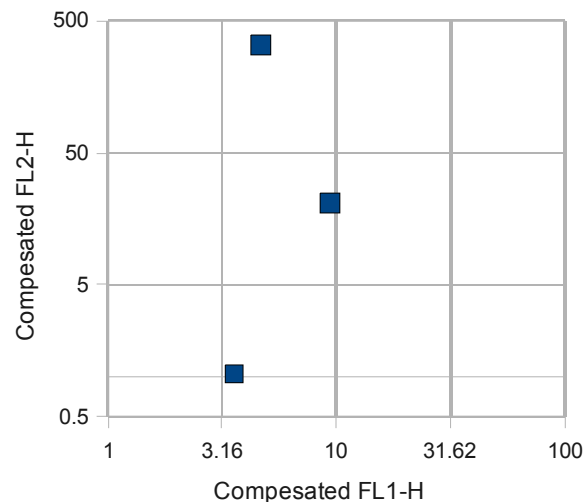

Figure 4: Example 1 - compensated values, displayed as suggested by the $PnD keyword.

Calibrated values:

```
Event          FSC-H          SSC-H          FL1-H          FL2-H
1              500            230            4.42           3.65
2              100            310            11.61          72.36
3              800            140            5.78           1130.98
Displayed min  -100           100            1.234          1.728
Displayed max  900            350            123.4          1728
```

Generally, there are two different approaches to compute the calibrated values; (1) after the compensation, (2) together with the compensation. These lead to the same results:

1. Carry out compensation calculations as shown above and then multiply the compensated values by the calibration coefficients.
2. Form a diagonal matrix D with rows and columns defined as for the spillover matrix, the appropriate conversion factors in the diagonal position, 1s for any other diagonal elements that are not covered by calibration, and 0s elsewhere. Multiply the inverse of the spillover matrix by the diagonal matrix D, i.e., $C = S^{-1} * D$. The row vector of calibrated values can be obtained as the result of matrix multiplication of the row vector of uncompensated scale values by matrix C.

Diagonal matrix D	FL1-H	FL2-H
FL1-H	1.234	0
FL2-H	0	3.456

Spillover inverse S^{-1}	FL1-H	FL2-H
FL1-H	1.03	-0.12
FL2-H	-0.27	1.03

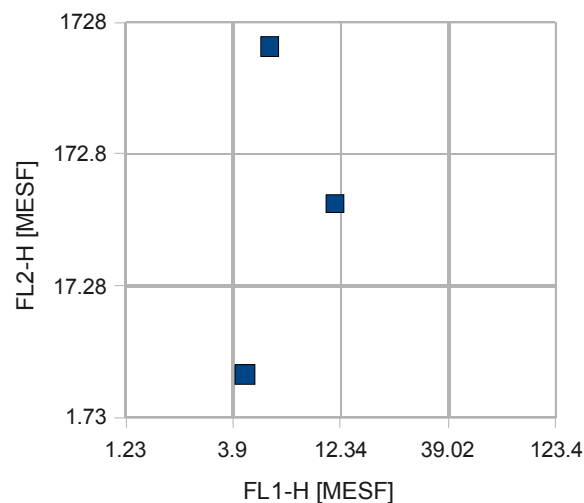| Calibration matrix C	FL1-H	FL2-H
FL1-H	1.27	-0.43
FL2-H	-0.33	3.57

Figure 5: Example 1 - values calibrated to the MESF units and displayed as suggested by $PnD.

Example 2: Floating point data type

Dataset header:

```
$MODE/L/     $DATATYPE/F/          $TOT/3/                $PAR/4/
$P1N/FSC-H/ $P1E/0,0/             $P1R/262144/           $P1B/32/ $P1D/Linear,-1000,100000/
$P2N/SSC-H/ $P2E/0,0/ $P2G/4/ $P2R/262144/               $P2B/32/ $P2D/Linear,0,100000/
$P3N/FL1-H/ $P3E/0,0/             $P3R/262144/           $P3B/32/ $P3D/Logarithmic,4,1/
$P4N/FL2-H/ $P4E/0,0/             $P4R/262144/           $P4B/32/ $P4D/Logarithmic,5,1/

$SPILLOVER/2,FL1-H,FL2-H,1,0.05,0.11,1/

$P3CALIBRATION/2.34,MESF/     $P4CALIBRATION/4.56,MESF/
```

Note that $PnE shall be 0,0 for all parameters when $DATATYPE F or D is used.

Channel values in file:

Event	FSC-H	SSC-H	FL1-H	FL2-H
1	1500	460000	-200.87	238545
2	82345.6	265000	20122.8	18456
3	-555.55	25878	30158.45	262144

- There are no restrictions on the minimum, neither on the maximum values allowed in the file. Specifically, values greater than $PnR are legal. The real range of data can be determined by searching for the minimum and maximum values among events in the data file.
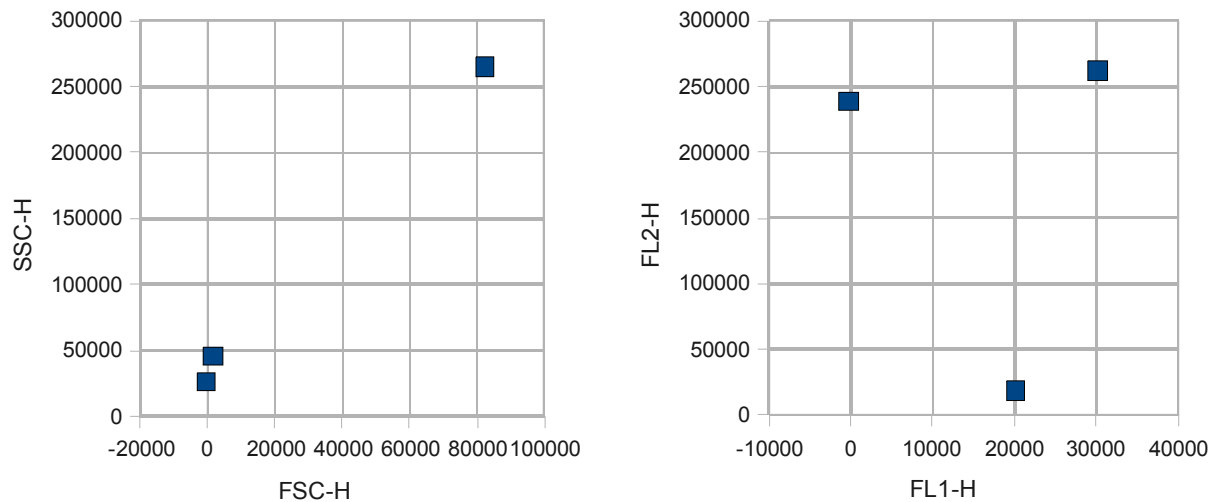
Figure 6: Example 2: channel values as in the FCS data file.

Scale values:

Event	FSC-H	SSC-H	FL1-H	FL2-H
1	1500	11500	-200.87	238545
2	82345.6	66250	20122.8	18456
3	-555.55	6469.5	30158.45	262144

- FSC-H and FL1-H: scale values equal channel values since $P1E/0,0/ and $P1G is not present
- SSC-H: scale values equal channel values divided by the value of the $P2G keyword

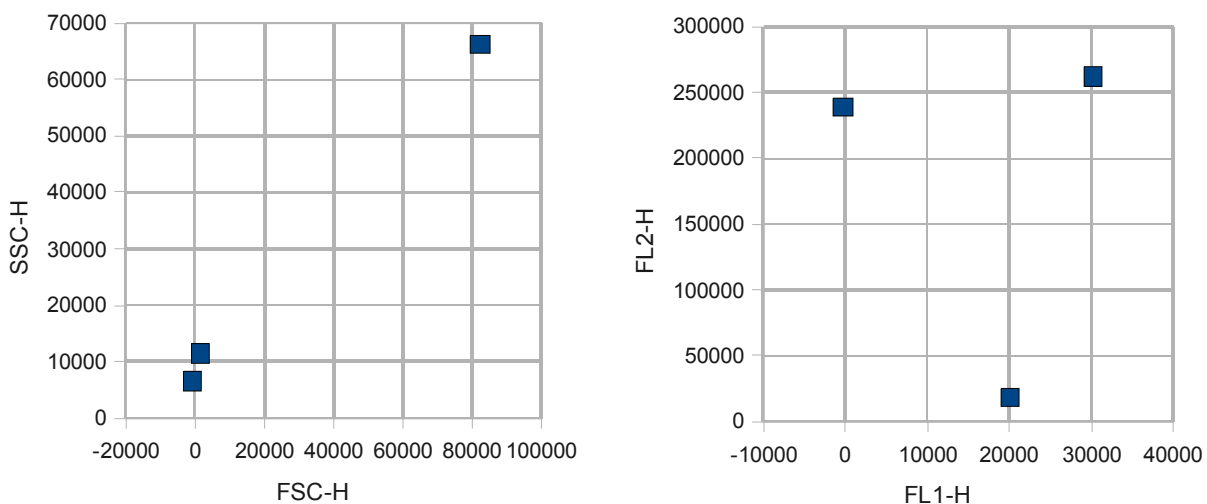Figure 7: Example 2 - scale values.

Displayed values (as suggested by $PnD):

```
Event              FSC-H         SSC-H        FL1-H        FL2-H
1                  1500          11500        -200.87      238545
2                  82345.6       66250        20122.8      18456
3                  -555.55       6469.5       30158.45     262144
Displayed min      -10000        0            1            1
Displayed max      100000        100000       316227.77    1000000
```

- The value of $PnD does not effect the actual values, it only recommends visualization scale for parameter n.
- In this case, we can see that the logarithmic display is unable to display negative values. Sophisticated analytical software would typically use another "log-like" display, such as logicle (1), hyperlog (2), or some others (3).
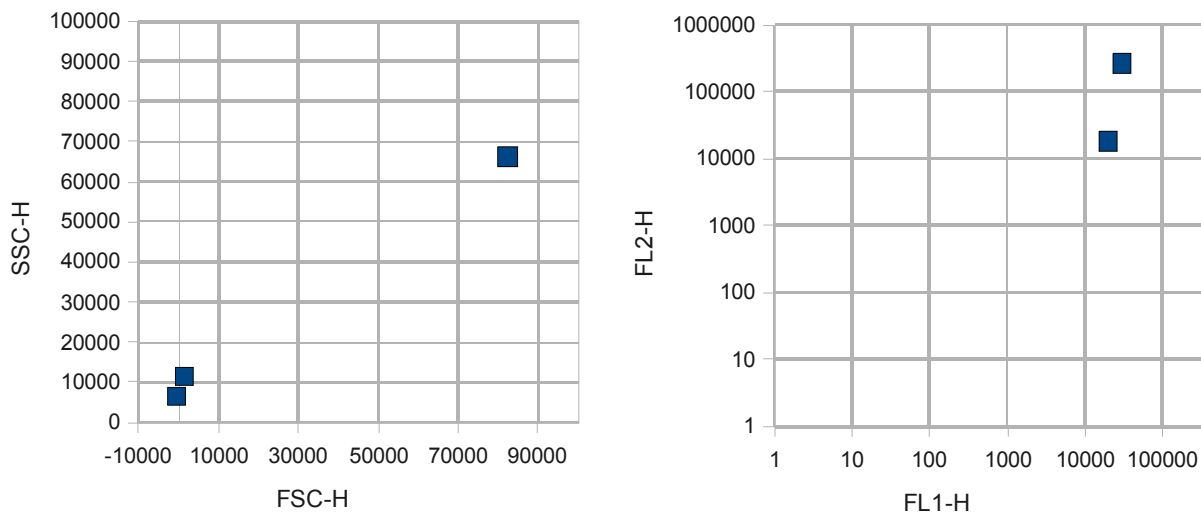

Figure 8: Example 2 - scale values displayed as suggested by the $PnD keyword.

Compensated values:

Spillover matrix S	FL1-H	FL2-H
FL1-H	1.0	0.05
FL2-H	0.11	1.0

```
Event              FSC-H         SSC-H        FL1-H        FL2-H
1                  1500          11500        -26587.05    239874.35
2                  82345.6       66250        18192.7      17546.37
3                  -555.55       6469.5       1329.92      262077.5
```

- The FSC-H and SSC-H parameters are not included in the spillover matrix, thus the values of FSC-H and SSC-H are not affected by the compensation.
- The row vector of compensated FL1-H,FL2-H values can be obtained as the result of matrix multiplication of the row vector of uncompensated FL1-H,FL2-H values by the inverse of the spillover matrix.
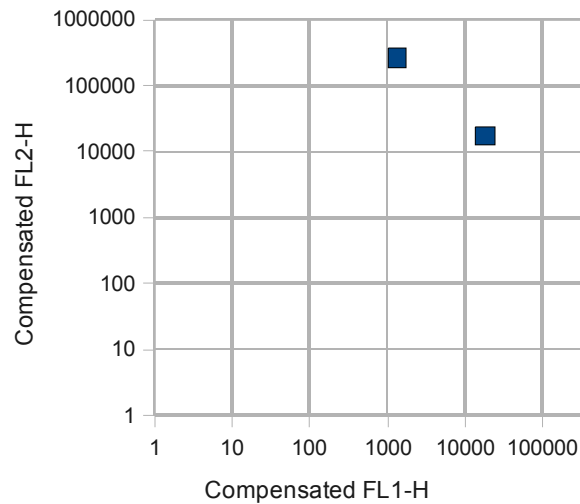
Figure 9: Example 2 - compensated values, displayed as suggested by the $PnD keyword.

Calibrated values:

Event	FSC-H	SSC-H	FL1-H	FL2-H
1	1500	11500	-62213.69	1093827.05
2	82345.6	66250	42570.92	80011.42
3	-555.55	6469.5	3112.02	1195073.42
Displayed min	-10000	0	2.34	4.56
Displayed max	100000	100000	739972.97	4560000

As described in example 1, there are two different approaches to compute the calibrated values; (i) after the compensation, (ii) together with the compensation. These lead to the same results.
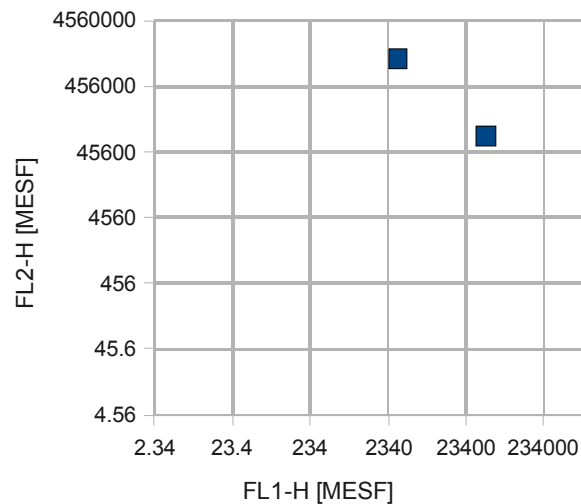

Figure 10: Example 2 - values calibrated to the MESF units and displayed as suggested by $PnD.

References:

1. Parks DR, Roederer M, Moore WA. A new "logicle" display method avoids deceptive effects of logarithmic scaling for low signals and compensated data. Cytometry A. 2006;69:541-551.

2. Bagwell CB. Hyperlog-a flexible log-like transform for negative, zero, and positive valued data. Cytometry A. 2005;64:34-42.

3. Battye FL. A Mathematically Simple Alternative to the Logarithmic Transform for Flow Cytometric Fluorescence Data Displays. Available at: http://www.wehi.edu.au/cytometry/Abstracts/AFCG05B.html.