Analyzing FACS data

We made the fluorescence observations on the FACS. The following steps were followed for extracting the CFP and YFP data; for separating CFP and YFP data from auto fluorescence and noise.

We have four variables x_1, x_2, x_3, x_4, corresponding to data obtained from FITC, PE and violet1 and violet2 filters respectively. We can always take one as parameter and write other three in terms of that parameter. Here, we use the variable x_1 for the YFP line because it has largest value for YFP. YFP is detected maximum in the FITC filter. Also, x_4 is used for CFP data because CFP has maximum value in violet2 filter. So for CFP we have equations like $x_1 = p_1 + q_1x_4$

$$x_2 = p_2 + q_2x_4$$
$$x_3 = p_3 + q_3x_4$$

For YFP our equations are

$$x_2 = p_1' + q_1'x_1$$
$$x_3 = p_2' + q_2'x_1$$
$$x_4 = p_3' + q_3'x_1$$

1) Getting CFP line by looking at the bright cells:

a) The data from the matrix is taken which has sufficiently high values in violet1 and violet2 filters. Let that matrix be F. Then we extract columns 3 through 6 from matrix F to obtain the data of the 4 filters. We do not need the first two columns as they just represent side scatter and forward scatter.

$$F = \begin{pmatrix} x_1^1 & x_2^1 & x_3^1 & x_4^1 \\ x_1^2 & x_2^2 & x_3^2 & x_4^2 \\ . & . & . & . \\ . & . & . & . \\ . & . & . & . \\ x_1^n & x_2^n & x_3^n & x_4^n \end{pmatrix}$$

b) We define a matrix A_1 such that A_1 = [ones (length(F),1) F(:,4)]. We choose fourth column because it represents the value in violet2 filter which has maximum value in CFP.

$$A_1 = \begin{pmatrix} 1 & x_4^1 \\ 1 & x_4^2 \\ . & . \\ . & . \\ . & . \\ 1 & x_4^n \end{pmatrix}$$

c) We define the matrix B_1 such that it contains all the rows and first three columns of F which contains the values of other three filters.

$$B_1 = \begin{pmatrix} x_1^1 & x_2^1 & x_3^1 \\ x_1^2 & x_2^2 & x_3^2 \\ . & . & . \\ . & . & . \\ . & . & . \\ x_1^n & x_2^n & x_3^n \end{pmatrix}$$

d) The equation becomes $A_1X_1=B_1$.

$$\begin{pmatrix} 1 & x_4^1 \\ 1 & x_4^2 \\ . & . \\ . & . \\ . & . \\ 1 & x_4^n \end{pmatrix} \begin{pmatrix} p_1 & p_2 & p_3 \\ q_1 & q_2 & q_2 \end{pmatrix} = \begin{pmatrix} x_1^1 & x_2^1 & x_3^1 \\ x_1^2 & x_2^2 & x_3^2 \\ . & . & . \\ . & . & . \\ . & . & . \\ x_1^n & x_2^n & x_3^n \end{pmatrix}$$

$$X_1 = \begin{pmatrix} p_1 & p_2 & p_3 \\ q_1 & q_2 & q_3 \end{pmatrix}$$ X_1 can be obtained by inverse(A_1)*B_1.

e) Define v_1 as a row matrix with last element 1 preceded by second row of matrix X_1.

$v_1 = [q_1\ q_2\ q_3\ 1]$

2) Getting YFP line.

a) This time the matrix F was formed when by taking data from the matrix which had sufficiently high value in FITC filter. Again we do not take the forward scatter side scatter values.

b) Matrix A_2 is constructed such that A_2= [ones (length(F),1) F(:,1)]. Here we choose the first column because it represents the value in FITC filter which has maximum value for YFP.

$$A_2 = \begin{pmatrix} 1 & x_1^1 \\ 1 & x_1^2 \\ . & . \\ . & . \\ . & . \\ 1 & x_1^n \end{pmatrix}$$

c) Now we define the matrix B_2 such that it contains all the rows and last three columns of B_2, dropping the column containing FITC filter value.

$$B_2 = \begin{pmatrix} x_2^{1} & x_3^{1} & x_4^{1} \\ x_2^{2} & x_3^{2} & x_4^{2} \\ . & . & . \\ . & . & . \\ . & . & . \\ x_2^{n} & x_3^{n} & x_4^{n} \end{pmatrix}$$

d) So $A_2X_2 = B_2$.

$$\begin{pmatrix} 1 & x_1^{1} \\ 1 & x_1^{2} \\ . & . \\ . & . \\ . & . \\ 1 & x_1^{n} \end{pmatrix} \begin{pmatrix} p_1' & p_2' & p_3' \\ q_1' & q_2' & q_2' \end{pmatrix} = \begin{pmatrix} x_2^{1} & x_3^{1} & x_4^{1} \\ x_2^{2} & x_3^{2} & x_4^{2} \\ . & . & . \\ . & . & . \\ . & . & . \\ x_2^{n} & x_3^{n} & x_4^{n} \end{pmatrix}$$

$X_2 = \begin{pmatrix} p_1' & p_2' & p_3' \\ q_1' & q_2' & q_3' \end{pmatrix}$ X_2 can be obtained by inverse(A_2)*B_2.

e) Define v_2 as a row matrix with first element 1 followed by second row of matrix X_2.
v2 = [1 q_1’ q_2’ q_3’]

Normalize v1 and v2.

Gram-Schmidt orthogonalization

Gram Schmidt orthogonalization is a general method used to construct an orthonormal basis from a set of linearly independent vectors. We already have two vectors v1 and v2 in the direction of CFP and YFP respectively. We define another two vectors v3 and v4 such that v3 = [0 1 0 0] and v4 = [0 0 1 0] and v1, v2, v3, v4 are linearly independent. Using Schmidt procedure we find orthonormal basis w1, w2, w3, w4. We proceed as follows.

w1 = v1

$$\text{w2} = \text{v2} - \frac{(\text{v2*w1})}{(\text{w1*w1}')}(\text{w1})$$

$$\text{w3} = \text{v3} - \frac{(\text{v3*w1}')}{(\text{w1*w1}')}(\text{w1}) - \frac{(\text{v3*w2}')}{(\text{w2*w2}')}(\text{w2})$$

$$\text{w4} = \text{v4} - \frac{(\text{v4*w1}')}{(\text{w1*w1}')}(\text{w1}) - \frac{(\text{v4*w2}')}{(\text{w2*w2}')}(\text{w2}) - \frac{(\text{v4*w3}')}{(\text{w3*w3}')}(\text{w3})$$

Normalize w_2, w_3, w_4.

We want vectors in CFP direction YFP direction and two orthogonal vectors perpendicular to plane of v1 and v2. Here w3 and w4 are vectors perpendicular to plane containing v1 and v2. So we define a matrix Amat such that

Amat = [v1’ v2’ w3’ w4’]

Take inverse of matrix Amat

Bmat = inverse(Amat)

If we take transpose of F our data matrix and hit it with Bmat we get the required values.

The values along v_1 correspond to CFP, the values along v2 corresponds to YFP. Whatever lies in w_3 w_4 plane has no component in CFP and YFP direction as it is perpendicular to v1 & v2

Suppose G = (Bmat*F’)’. The matrix G contains our required CFP and YFP value. The first column of our matrix G gives CFP values. The second column gives YFP values.

We calculate the error from the data of third and fourth column.

error = $\sqrt{G(:,3).\hat{}2 + G(:,4).\hat{}2}$

We can plot error/signal v/s signal plot and put suitable cut offs.