

INSTRUCTIONS FOR COURSE WORK 10: HCS ANALYSIS

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1. Purpose of the work

The aim of this work is to show how to use various data visualization and analysis tools.

2. Background

For the analysis of large data sets, statistical methods have to be used. Also, visualization of the data is often important, as it is difficult to detect meaningful changes in large numerical data sets. Also, comparison of numerical data is much more difficult than comparison of graphically presented data.

3. Software and data used

Software: Cellomics Toolbox (vHCS View); DecisionSite Spotfire

Data: Data generated in the course work 7 (HCS) will be analyzed.

4. Task

You have two tasks. First, you will use visual inspection of your data to estimate how well your threshold value set in work 7 worked. Second, you will analyze the shape of the nuclei and see if there are any differences between the transfected and non-transfected cells.

The shape of the nuclei is analyzed using P2A, which is the ratio of the perimeter squared to the area times 4 pi. This is a measure of roundness of the object. For a circle, the P2A value is 1, and irregular shaped objects have values >1 .

5. Instructions for performing the work

1. Find your scan in the scan list and double-click on it. A new window will pop up. Select in the well feature list ValidObjectCount by clicking on the text and then on the single arrow button pointing to the right (Fig. 1). In the same way, select %AvgIntenRespondersCh2. Click ok. If you make a wrong selection, use the arrow pointing to the left to cancel the selection (select first the feature).

2. Again, a new window will appear (Fig. 2). On the output parameter list on the left (red arrow in Fig. 2), select %AvgIntenRespondersCh2 by clicking on the text. Now you will see the percentage of transfected cells in each scanned well. Write down the results.

3. Select the scanned wells by dragging the mouse over them in the spreadsheet on the right (the selected wells are shown in blue/black, see Fig. 2). In the main menu, select Spreadsheet (red circle in Fig. 2), then Cell details. Select AvgIntenCh2, and click ok.

4. In the new window, there is a graph showing the number designated for each selected cell versus its average intensity in channel 2 (Fig. 3). Are there any outliers

(cells whose intensity is significantly higher than in other cells)?

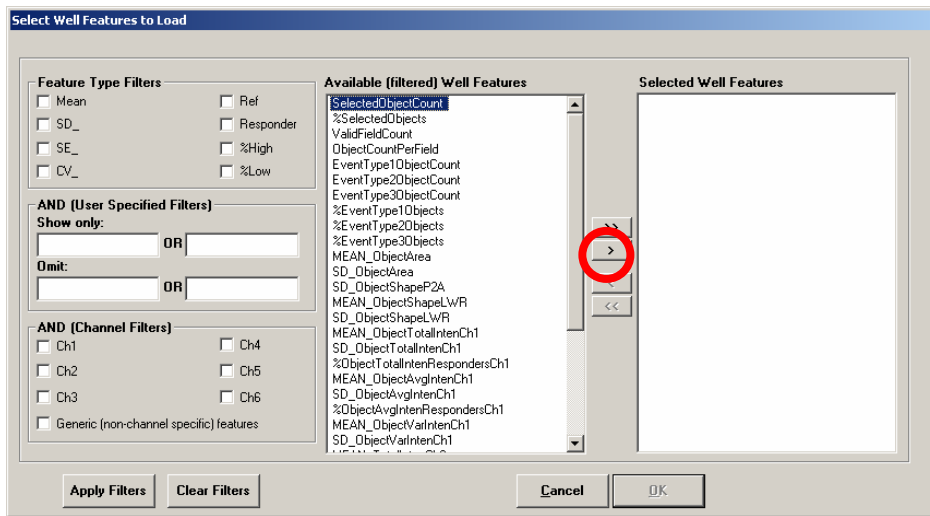


Figure 1. Well features selection. Red circle: selection button.

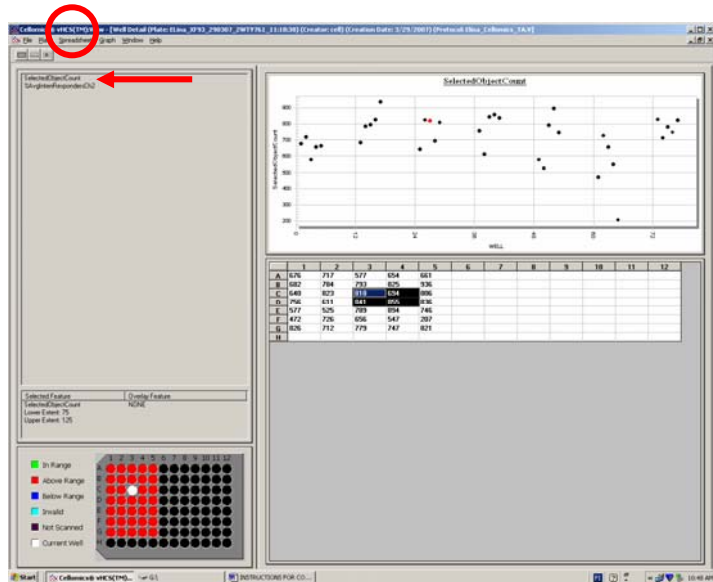


Figure 2. Well detail window

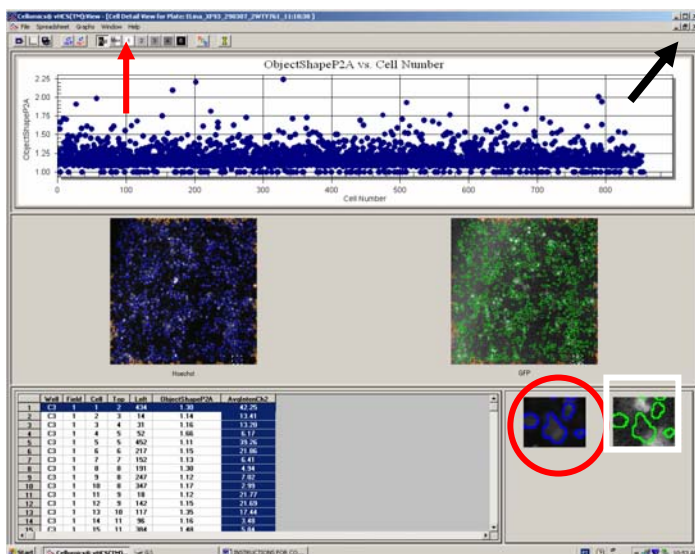


Figure 3. Cell detail window

5. On the lower left, there is a spreadsheet showing for each cell included in the analysis the well and field where it is located, coordinates, and average intensity in channel 2. Click on the column name “AvgIntenCh2” (the column should turn blue). Then right-click on it and select “Sort Ascending” in the pop-up selection. Wait until the software has performed sorting of the values.

6. Scroll down until you reach the average intensity value that matches the threshold value you used for “AvgIntenLevelHighCh2” in course work 7. Select a cell (by clicking on it) that has an intensity value matching the threshold value. The software will show you the images of that cell on the right for channels 1 (red circle in Fig. 3) and 2 (white square in Fig. 3). The cell in question is always in the center of the image. Does the cell look positive or negative?

7. The screen displays only 255 different gray-scale values (8-bit), whereas the camera used can record 4095 gray-scale values (12-bit). Although you cannot see all the 4095 different gray-scale values at the same time on the screen, you can inspect a limited range of them; if you press button marked 1 on the top panel (red arrow in Fig. 3), you will see values 0-255 out of the 4095. If the image looks too dark or too bright, select the next range by pressing button 2 (bits 2-9). In this way, you can look at the raw data without any scaling transformations. If you want to look at the images converted into an 8-bit scale (this is the default setting), click Full button.

8. Continue looking cells around the threshold value used; some that have lower intensity value, and some that have an intensity value just above the threshold. Do any of the cells in negative control wells have intensity values above the threshold? Are you satisfied with the threshold value chosen, or do you feel it should be readjusted? If so, should it be lower or higher? What factors made it difficult to determine the threshold value correctly in work 7?

9. Close the cell detail window by clicking on the lower x-button in the top right corner of the window (black arrow in Fig. 3). You should be back in the well detail window. In the main menu, select Spreadsheet/Spotfire IT. In the new window, select ObjectShapePA2 and click ok. Wait until the Spotfire software opens, showing your data (Fig. 4).

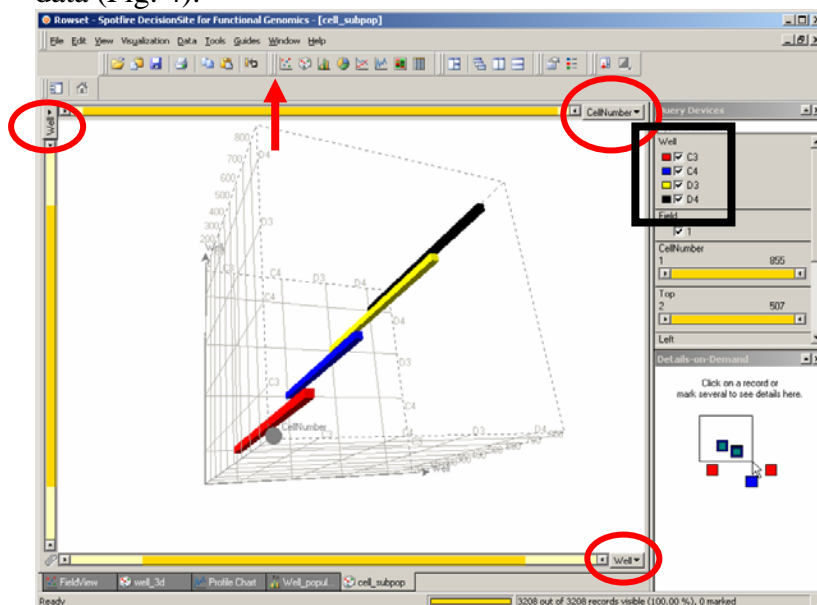


Figure 4. Spotfire cell_subpop window.

10. Select cell_subpop in the bottom menu. Select the x, y, and z axes (red circles in Fig. 4) so that one of them will be well, one cell number, and one ObjectShapeP2A (click on the axis button, and make your selection in the pop-up menu). Try different ways of defining the axes. You can change the perspective angle by moving the mouse over the graph and right-clicking. Then select Properties in the pop-up menu, and click on the 3D tab (Fig. 5). Dragging the Perspective slider (red oval in Fig. 5) will change the viewing angle. When done, close the window by clicking on the x button in the top right corner of the window.

Are there any differences in roundness when you compare the non-transfected and transfected cells? Try to use the software for the analysis of possible correlation between the shape and intensity of each cell. Do you find any? You can also select just one well for the analysis by unmarking the others in the well list (black square in Fig. 4). Also, you can use other visualization tools such as scatter plot (red arrow in Fig. 4).

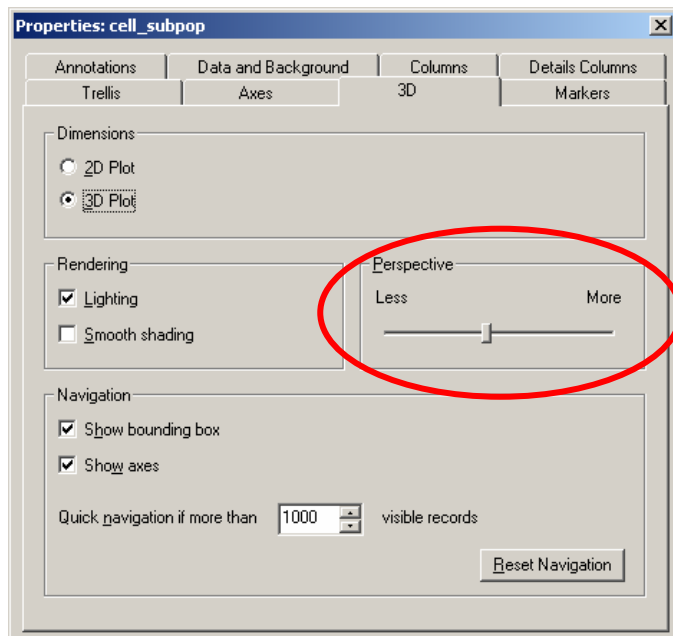


Figure 5. Properties/3D window.