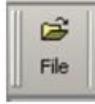**Flow Cytometry**

MODFIT LT TUTORIAL

AUTHORS: Andreia Santos
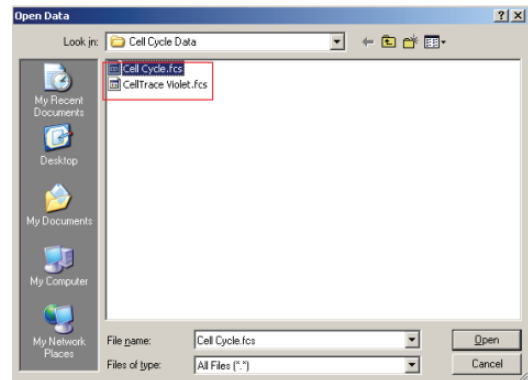
Mariana Fernandes

Rute Gonçalves

APPROVAL: Mariana Fernandes

## Cell cycle analysis using ModFit LT software v3.2

1. Launch ModFit LT software and click the Open File icon in the upper left. Alternatively, click File → Open Data from the dropdown menu on the command bar.


2. Navigate to the folder where the FCS 3.0 data files are located, select the file, and click Open.

3. The option box, as shown below, will open. Select the parameter used for the cell cycle stain. Select OK.
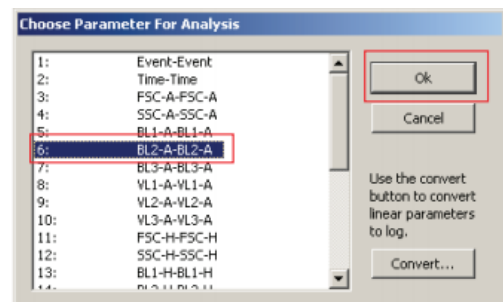
4. A second dialog box will open to prompt the user to define gates if a specific population of cells is of interest. **Remove debris and aggregates** from further analysis by creating two gates. To create the first gate, click Enable Gate 1 and select the Define Gate 1 button. Selected gates are listed as shown below.
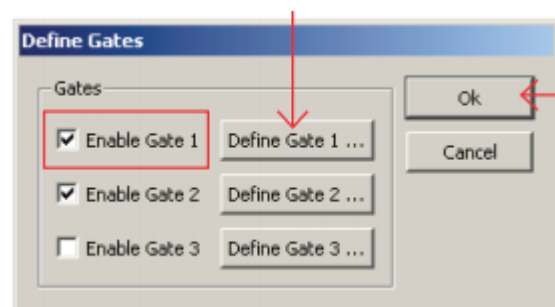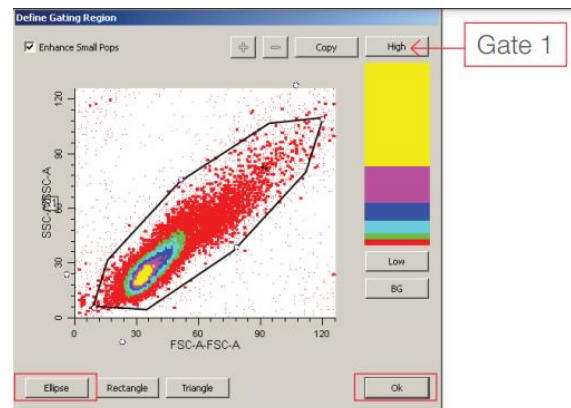
5. Select FSC-A for the x-parameter and SSC-A for the y-parameter and select OK. This will open up the Define Gating Region dialog box as shown below.
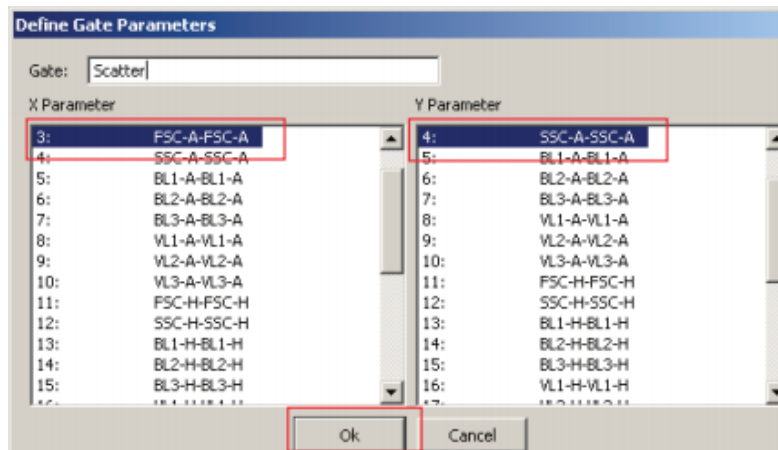

6. Select the type of gate using the buttons at the bottom and draw a region around the population of interest. Select OK.

7. Additional gates can be defined by selecting the Enable Gate 2 followed by selecting Define Gate 2. This will open the Define Gate Parameters for the second gate.

Tip: The width parameter on the software is collected directly from the instrument and will have values that will not scale correctly in ModFit LT software. We suggest that you use the area and height parameters to gate singlet cells if needed.
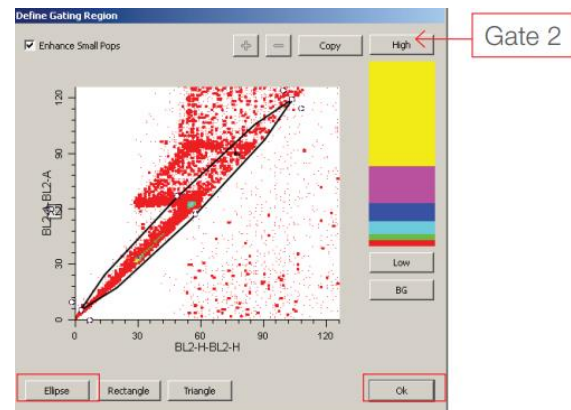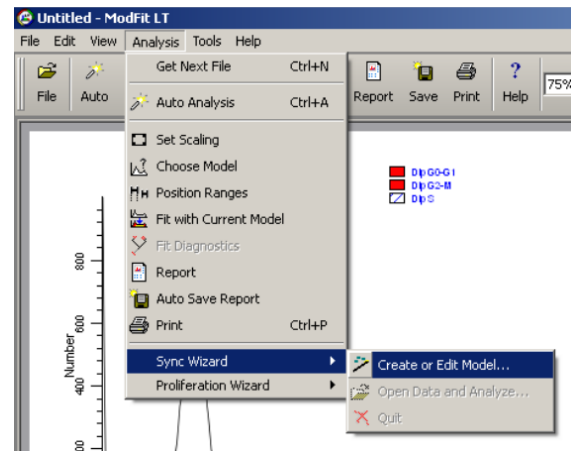

8. Click OK on the Define Gates dialog box, and a plot will open with the desired histogram displayed.

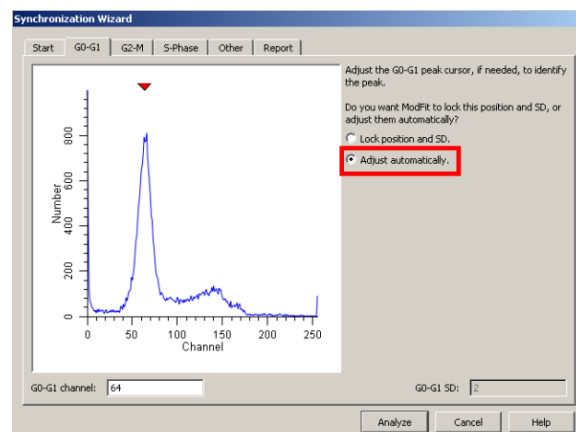**9. You have your optimized model for cell cycle analysis.**

10. For further optimization and modeling of data:

From the dropdown menus at the top, select Analysis → Sync Wizard → Create or Edit Model
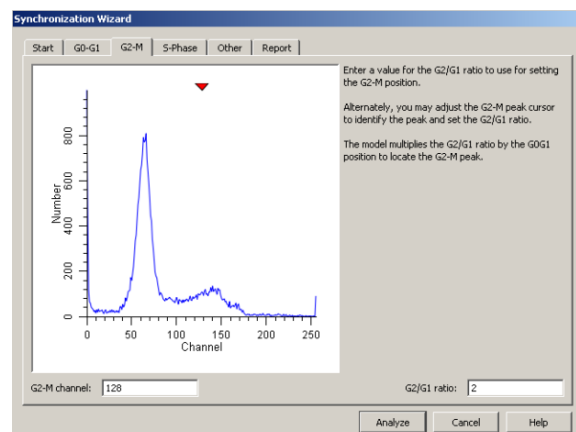

Start by letting the G0G1 peak position and SD adjust automatically.

If required, adjust the G2-M position

Maintain the S-phase default options to start, which are the simpler models.
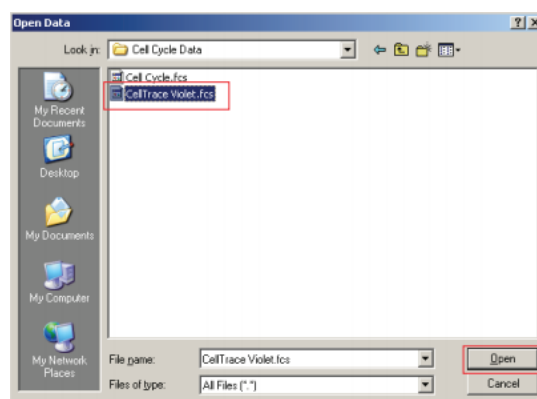
## Cell tracing and proliferation analysis using ModFit LT software v3.2

1. Launch ModFit LT software and click the Open File icon in the upper left. Alternatively, click File → Open Data from the dropdown menu on the command bar.
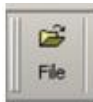
2. Navigate to the folder where the FCS 3.0 data files are located, select the file and click Open.

3. The option box, as shown below, will open. Select the parameter appropriate for the stain used in cell proliferation and tracing. Click the Convert button in the lower right corner of the dialog box as shown below. Click OK.
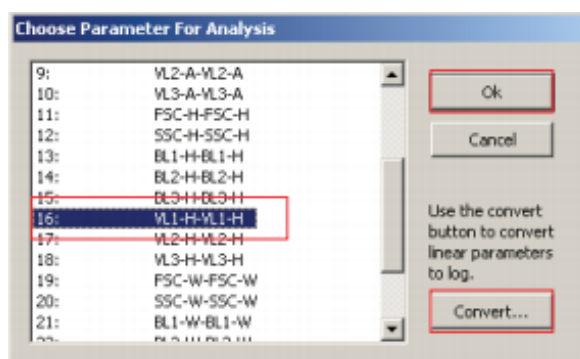
b. Ensure that all fluorescence parameters measured during acquisition are selected in the Log conversion option dialog box. Select 4-Decades from the dropdown menu at the top of the dialog box denoted Number of decades, and click OK. This will convert the linear fluorescence data collected by digital cytometers like the BD LSR Fortessa to log scale for analysis.
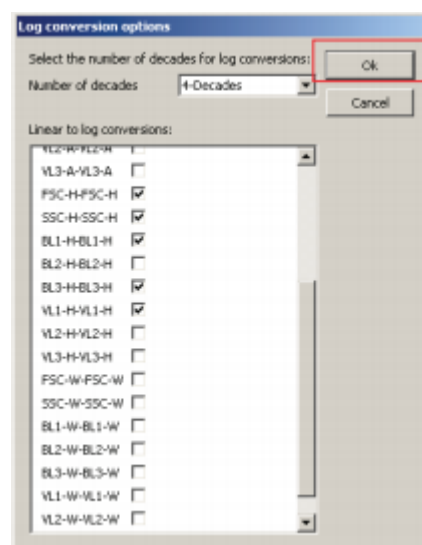
4. Select OK on the Choose Parameter For Analysis option box from step 3. This will open up the Define

Gates options. **Remove debris and aggregates** from further analysis by creating two gates.
See steps 4–7 in "Cell cycle analysis using ModFit LT software 3.2" above (pages 2-3) for descriptions of defining gates.

5. Click OK on the Define Gates dialog box, and a plot will open with the desired histogram displayed.

6. **You have your optimized model for cell proliferation analysis.**

7. For further optimization and modeling of data:

From the dropdown menus at the top, select Analysis → Proliferation Wizard → Create or Edit Model