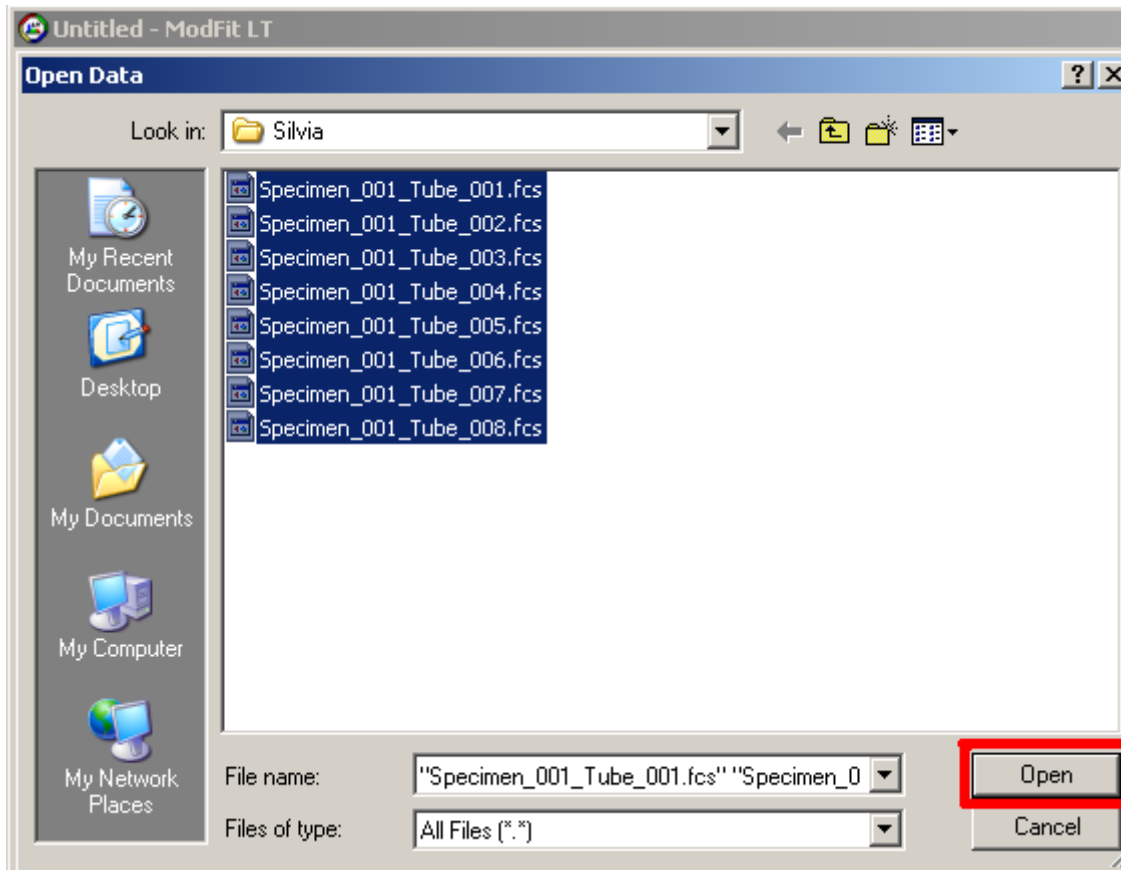
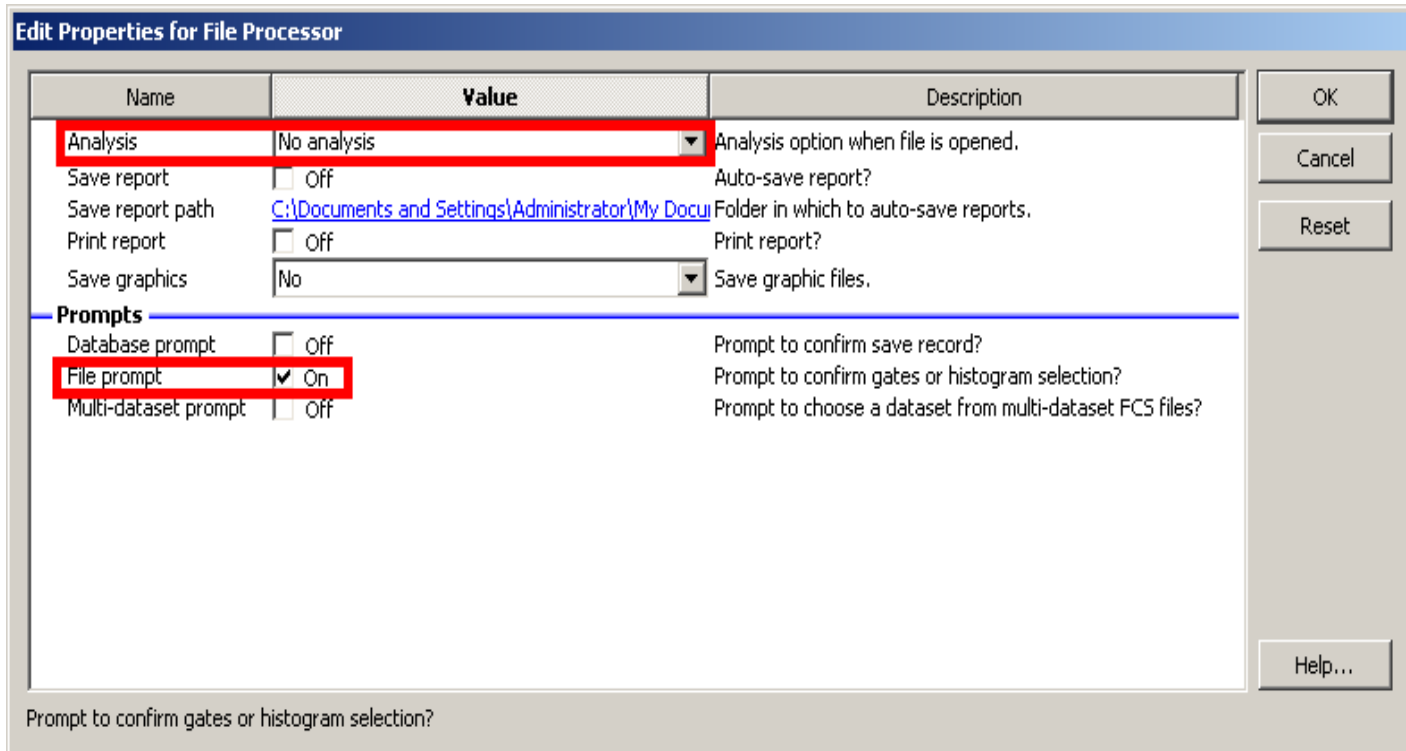


1. Start by choosing all the files to progress in batch mode.

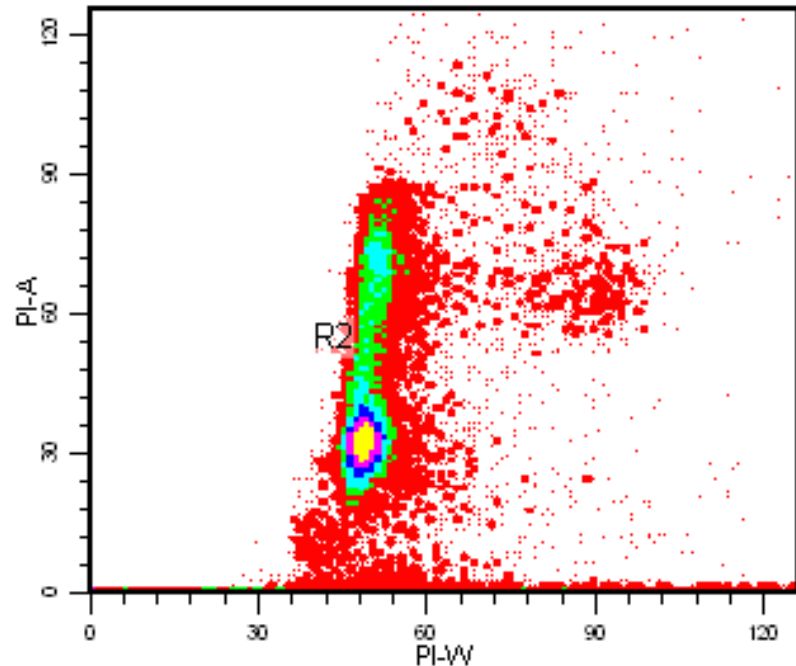
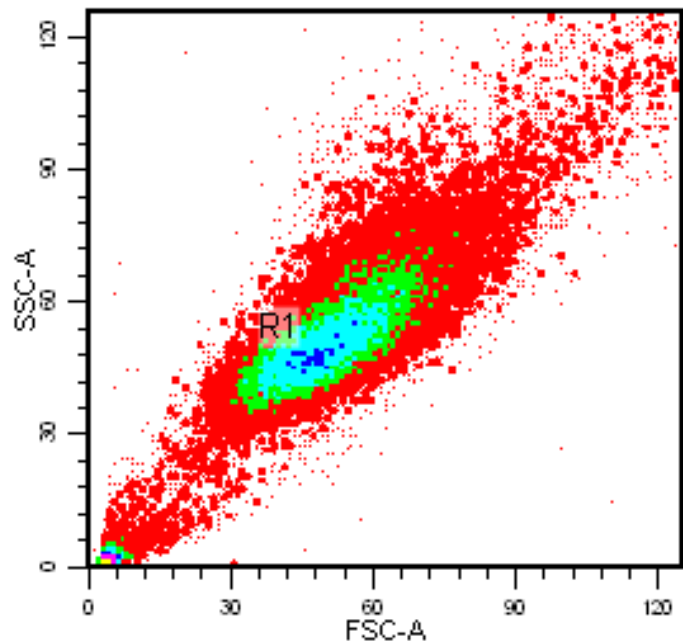


2. Set the batch controls to not analyze but with file prompt on. This allows you to open files and alter the gates for analysis but will not try to fit the data. This lets you just look at the unfitted histograms first.

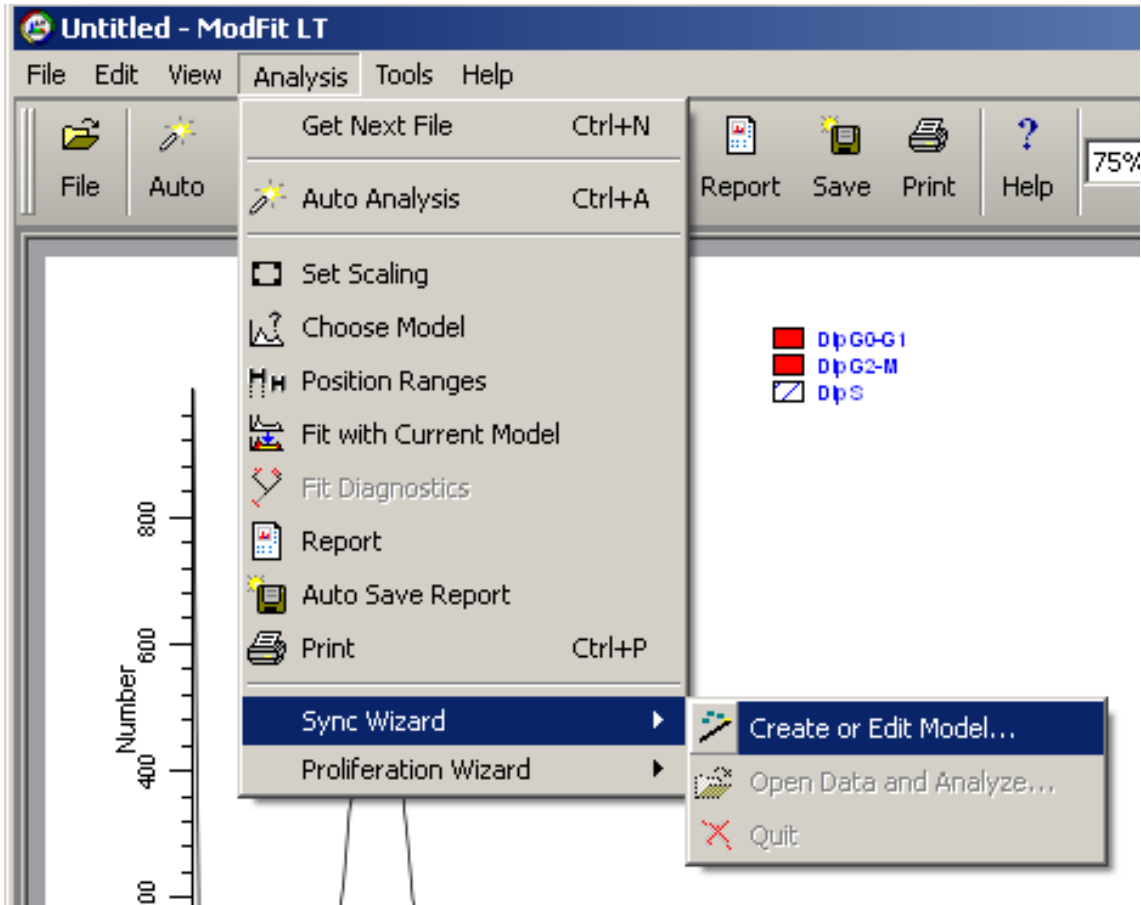


3. Choose the parameter for analysis (PI-A).

4. Select the first two gates and create them wide open (to cover the whole plot). This is so you can see the general position of populations and how they change between files. We can change these gates later if needed.



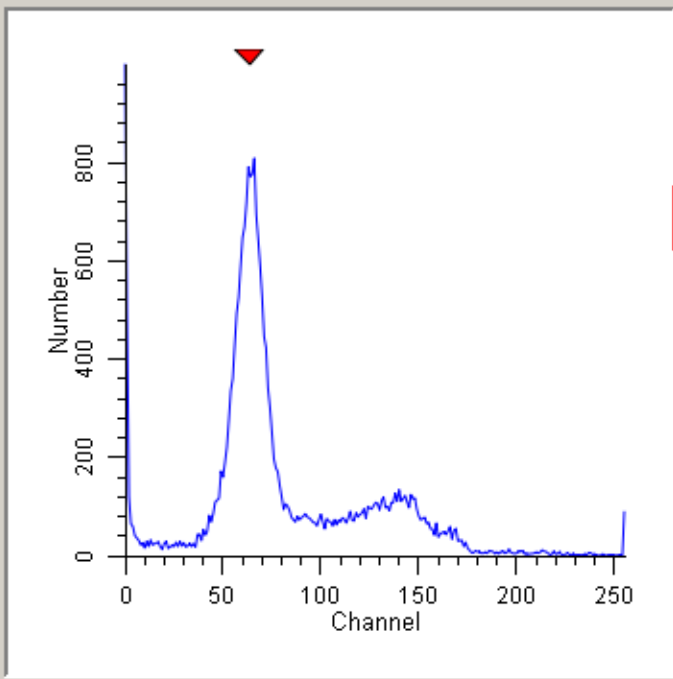
5. We can now set up the Synch Wizard.



It's good to start by letting the G0G1 peak position and SD adjust automatically.

Synchronization Wizard

Start G0-G1 G2-M S-Phase Other Report



Adjust the G0-G1 peak cursor, if needed, to identify the peak.

Do you want ModFit to lock this position and SD, or adjust them automatically?

Lock position and SD.

Adjust automatically.

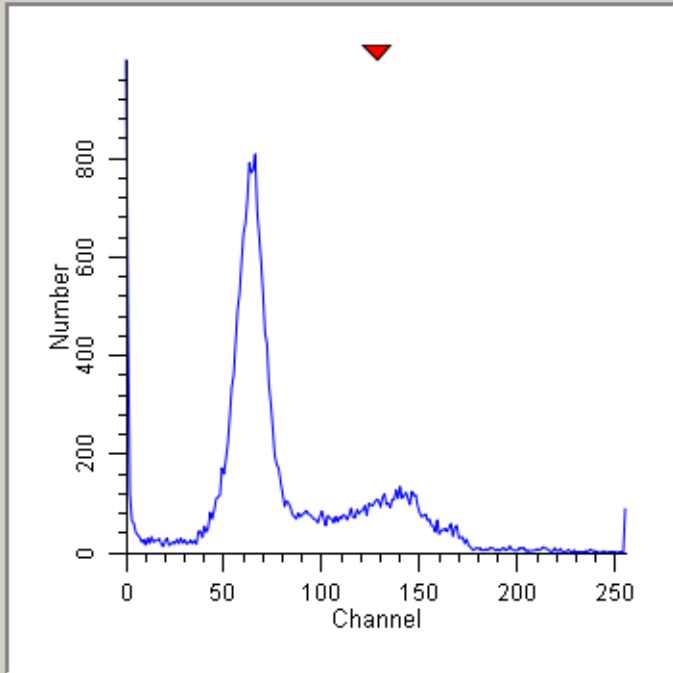
G0-G1 channel: G0-G1 SD:

Analyze Cancel Help

G2M position looks good so no need to change this.

Synchronization Wizard

Start | G0-G1 | **G2-M** | S-Phase | Other | Report



Enter a value for the G2/G1 ratio to use for setting the G2-M position.

Alternately, you may adjust the G2-M peak cursor to identify the peak and set the G2/G1 ratio.

The model multiplies the G2/G1 ratio by the G0G1 position to locate the G2-M peak.

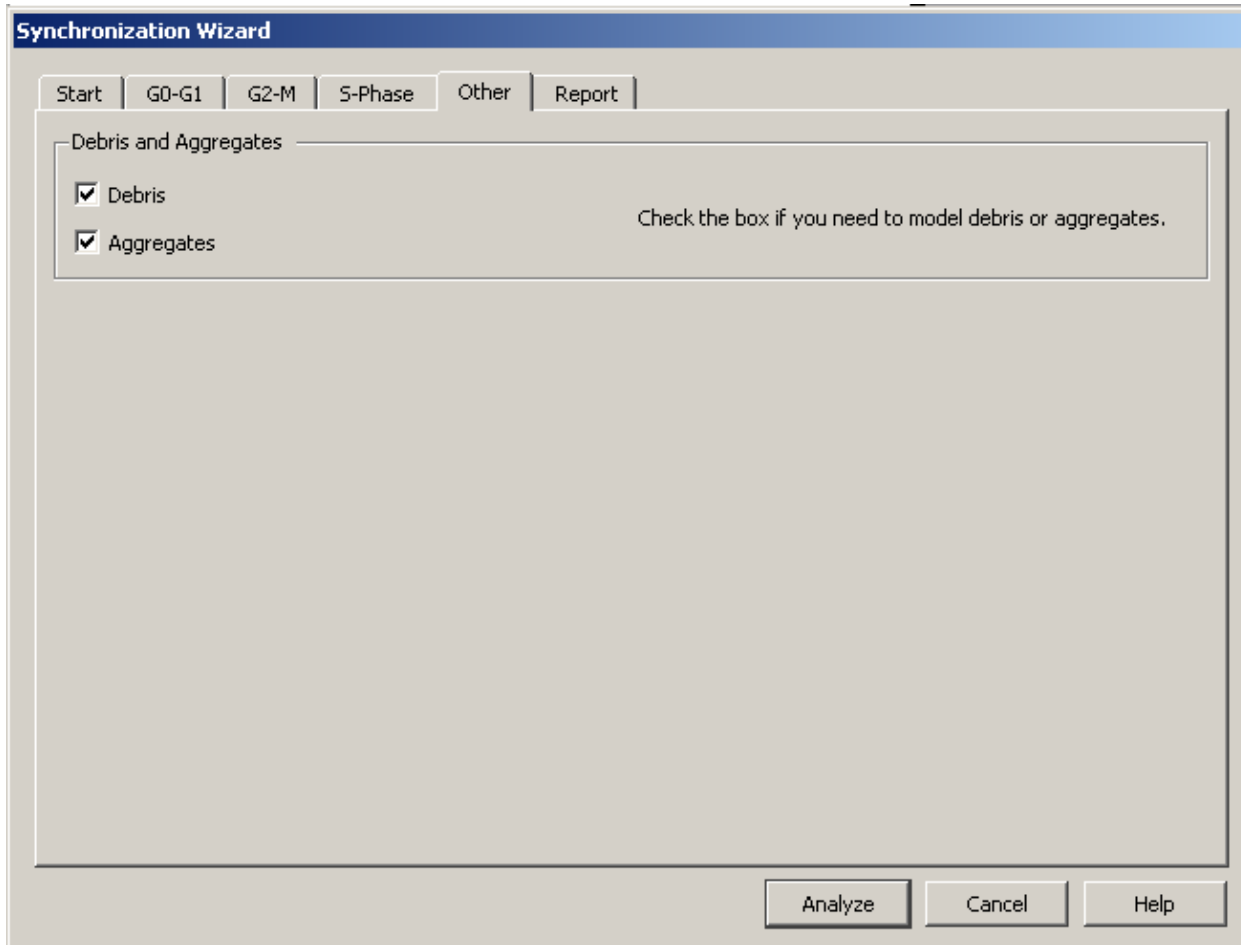
G2-M channel:

G2/G1 ratio:

Analyze | Cancel | Help

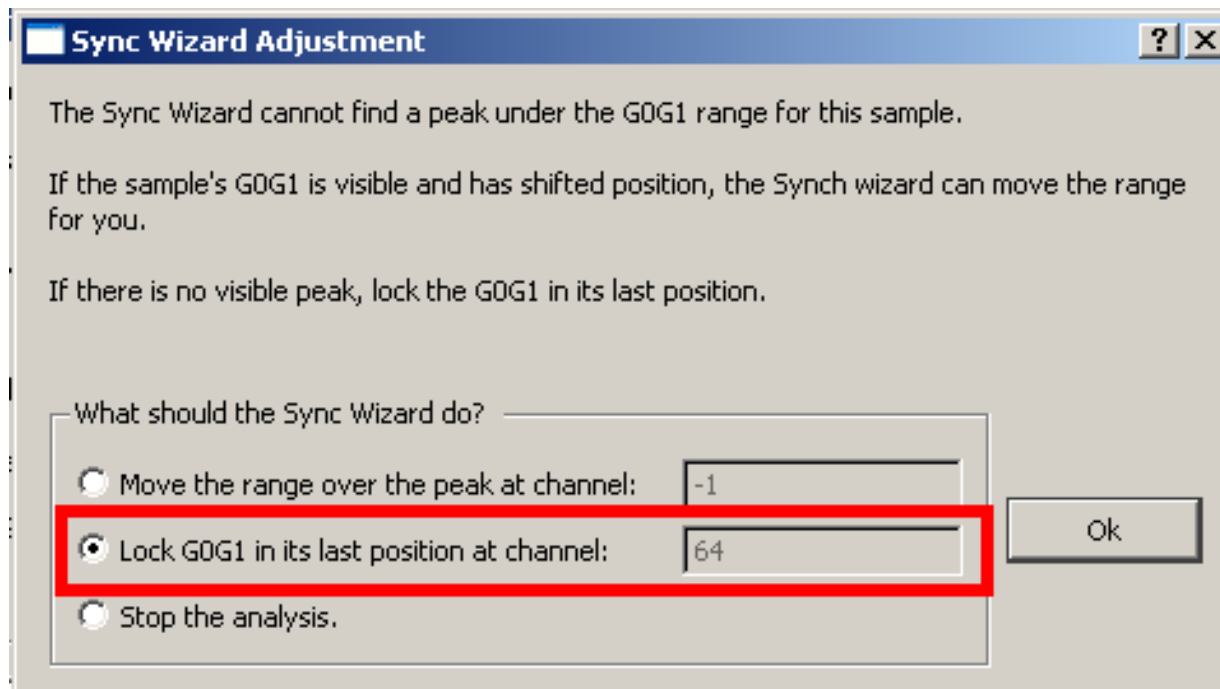
It's also good to maintain the S-phase default options to start, which are the simpler models.

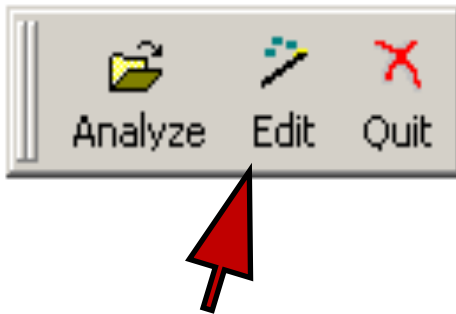
Under the 'Other' tab you can start with having the Debris and Aggregates checked.



Usually there is no reason to change ModFit's defaults so this is why we want to start with them first.

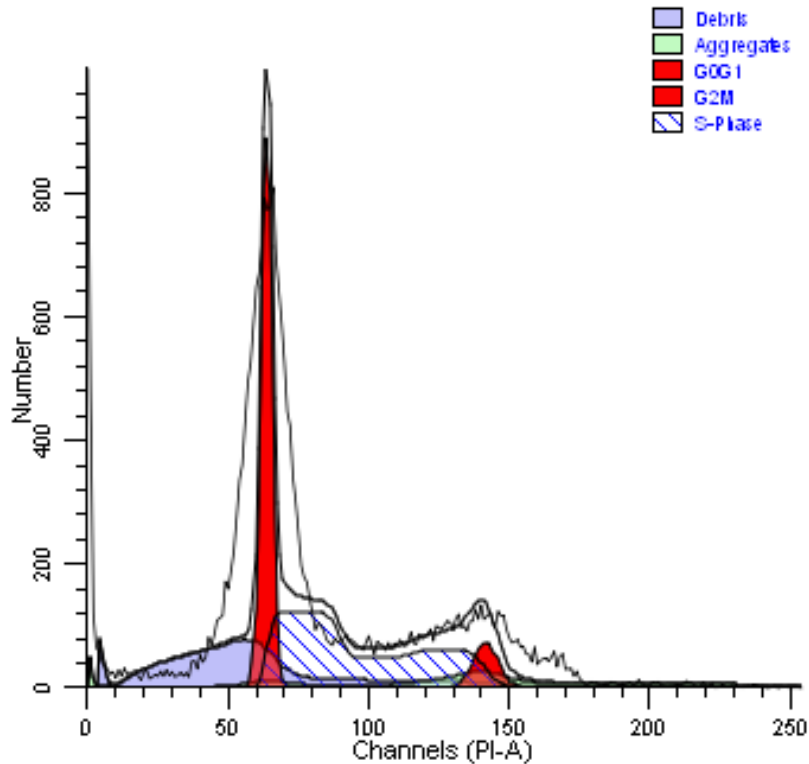
6. This first file produces the following error, telling us that the G0G1 position has shifted beyond the expected amount and wants you to tell it what to do next. If the new value were close to the original position (around 50 or so), then you can let it move to the new position. However, a position of -1 does not make sense, so we will choose to lock the G0G1 position. Once the model is locked, it will stay locked until you change it.





- In some instances you will see subsequent files that have a very distinct G0G1 peak that has shifted to the right or left from this locked position so you can click the model edit button to bring up the edit dialog again.
- If it appears to have shifted back to the original, expected location then you can choose to re-select the Adjust automatically mode. Otherwise, you can just drag the arrow over the new G0G1 position.

6. If the normal control for synch experiments has a wide CV, then ModFit will be unable to go through the Synch Wizard properly and you will get high RCS values back.



Sync Wizard Model

File: Specimen_001_Tube_001.fcs
Date acquired: 16-FEB-2011
Date analyzed: 22-Mar-2011

G0G1:
39.55 % Mean: 64.00
CV: 3.12 %

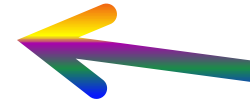
G2M:
7.07 % Mean: 142.00
G2/G1: 2.22

S-Phase:
53.38 % Mean: 96.58

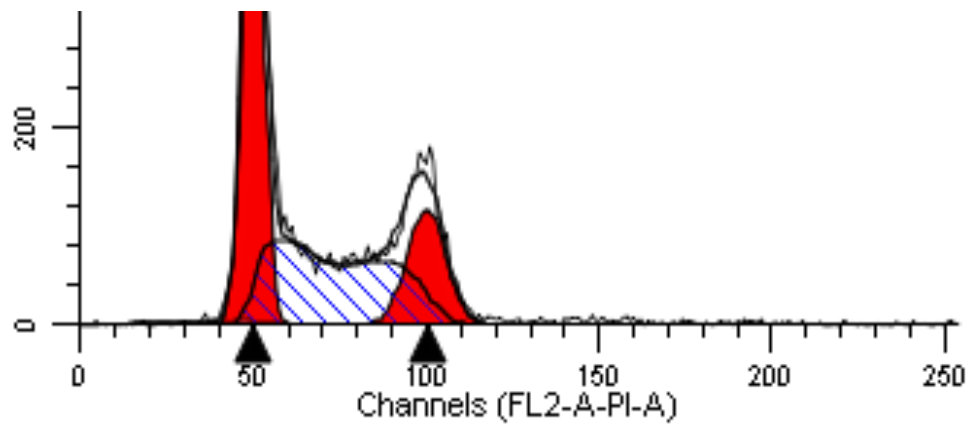
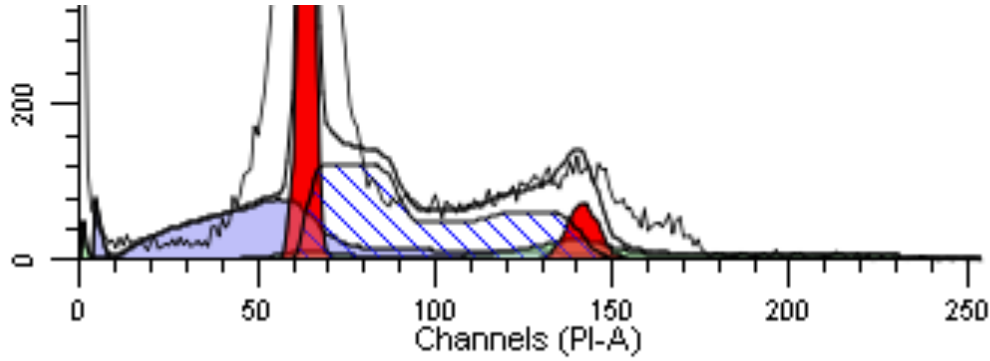
Compartment 1: 27.83 %
Compartment 2: 11.10 %
Compartment 3: 14.45 %

Debris: 24.68 %
Aggregates: 10.82 %
Modeled Events: 17674

RCS: 24.914

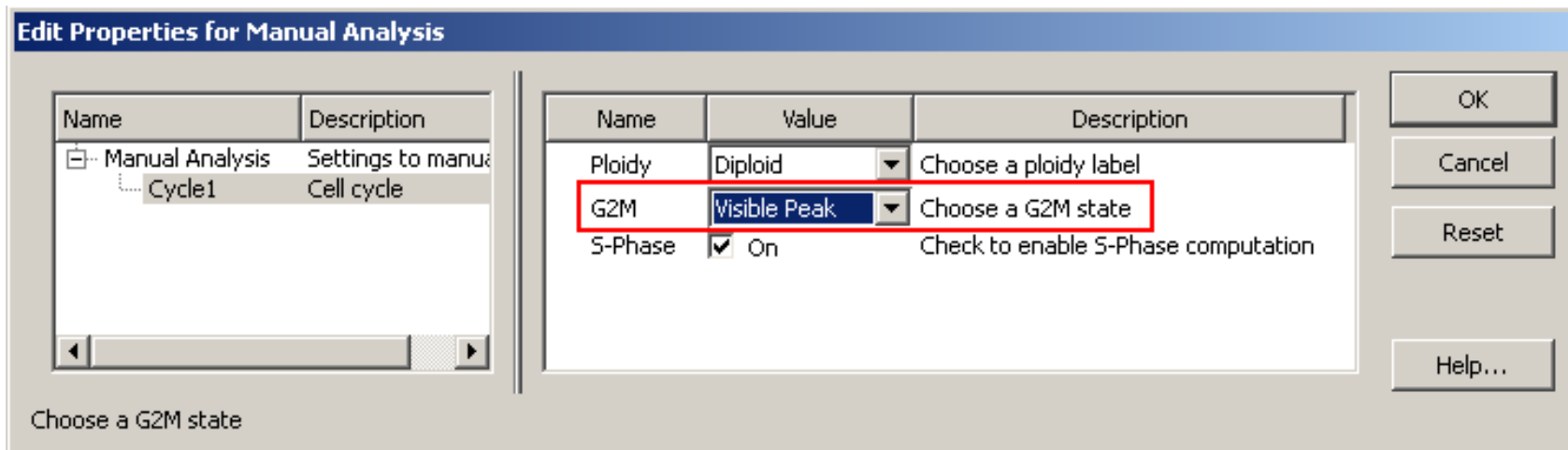


In this case you will have to proceed manually.



Verity also mentioned that if there no black triangles present underneath the G0G1 and G2M peaks then this is another indication of CV's that are too high to use Synch Wizard with.

6. You will have to exit out of Synch Wizard and click on the Mod icon. From here you can tell ModFit to look for G2M as a visible peak.

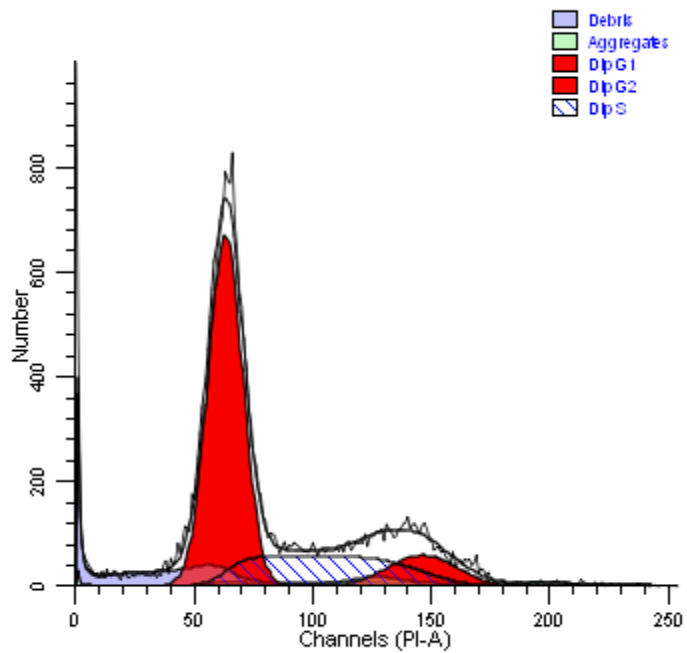


Keep Auto aggregates and debris on.

Edit Properties for Manual Analysis

Name	Description
Manual Analysis	Settings to manually a...
Cycle1	Cell cycle

Name	Value	Description
AutoDebris	<input checked="" type="checkbox"/> On	AutoDebris Compensation
AutoAggregates	<input checked="" type="checkbox"/> On	AutoAggregate Compensation
Apoptosis	<input type="checkbox"/> Don't Model	Check to model apoptosis peak
Linearity	2.00	G2/G1 ratio (1.50 to 2.50)
Standards	0	Number of internal standards
Number of cycles	1	Number of cell cycles in sample
Model templates	Diploid	Select a model template
Range positions	Compute range positions	Choose an option.



File analyzed: Specimen_001_Tube_001
 Date analyzed: 22-Mar-2011
 Model: 1DA0n_DSF
 Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %
 Dip G1: 63.94 % at 63.49
 Dip G2: 11.36 % at 146.48
 Dip S: 24.70 % G2/G1: 2.31
 %CV: 11.61

Total S-Phase: 24.70 %
 Total B.A.D.: 10.51 %

Debris: 13.32 %
 Aggregates: 5.53 %
 Modeled events: 23868
 All cycle events: 19371
 Cycle events per channel: 231

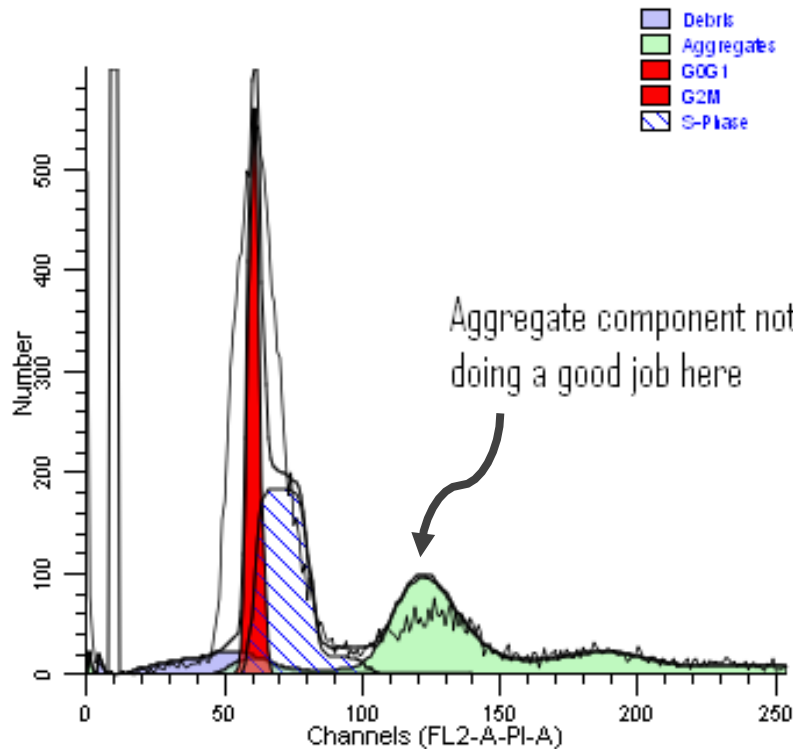
RCS: 1.958

RCS value
 went from
 24.914
 down to
 1.958!

Synch Wizard

1. Open all the files in a batch, make the gates wide open, open Synch Wizard.
2. Set the location for G0G1 and let it adjust the position automatically (the default). Check and make sure G2M position looks good. Let the S-phase options remain the default and check the auto-aggregate and debris boxes. Click Analyze.

3. Sometimes the synch wizard doesn't do a good job at calculating aggregates automatically.



Sync Wizard Model

File: MK_030811.002

Date acquired: 08-Mar-11

Date analyzed: 22-Mar-2011

G0G1:

40.87 % Mean: 61.00

CV: 3.28 %

G2M:

0.00 % Mean: 122.00

G2/G1: 2.00

S-Phase:

59.13 % Mean: 73.08

Compartment 1: 53.78 %

Compartment 2: 5.35 %

Compartment 3: 0.00 %

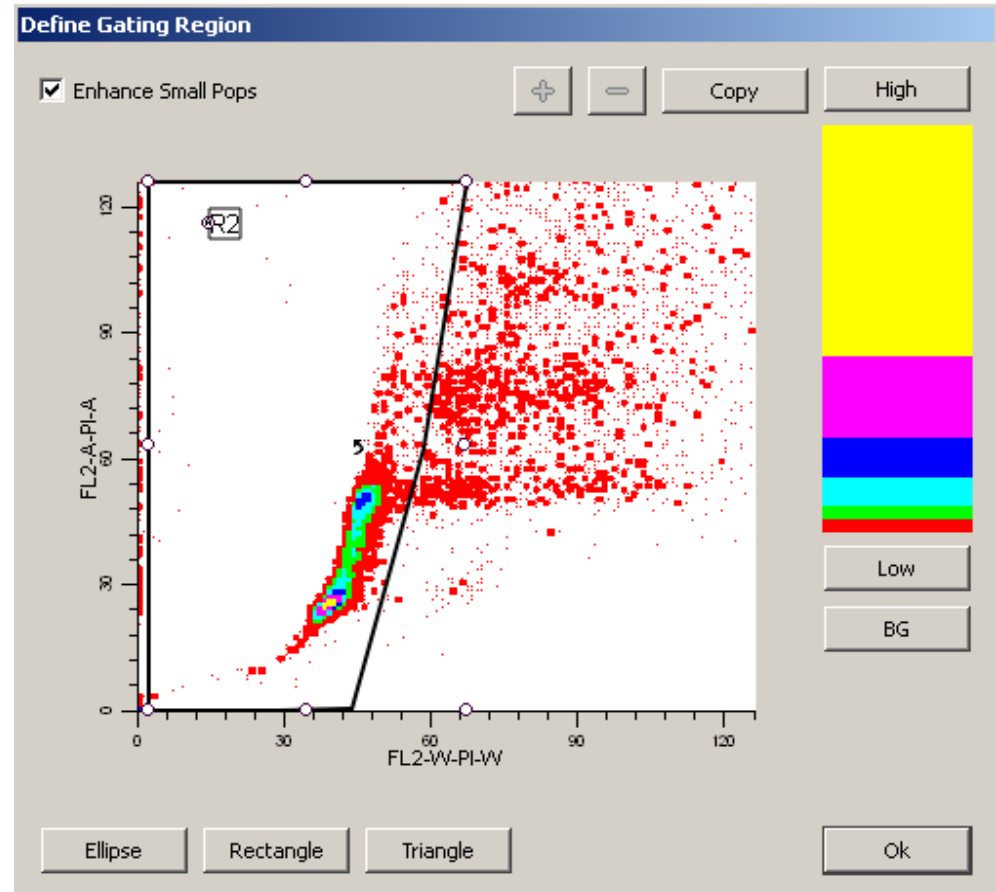
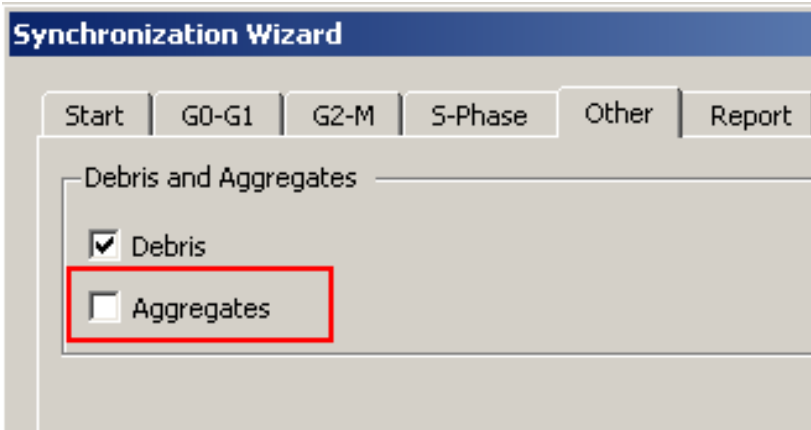
Debris: 8.74 %

Aggregates: 37.67 %

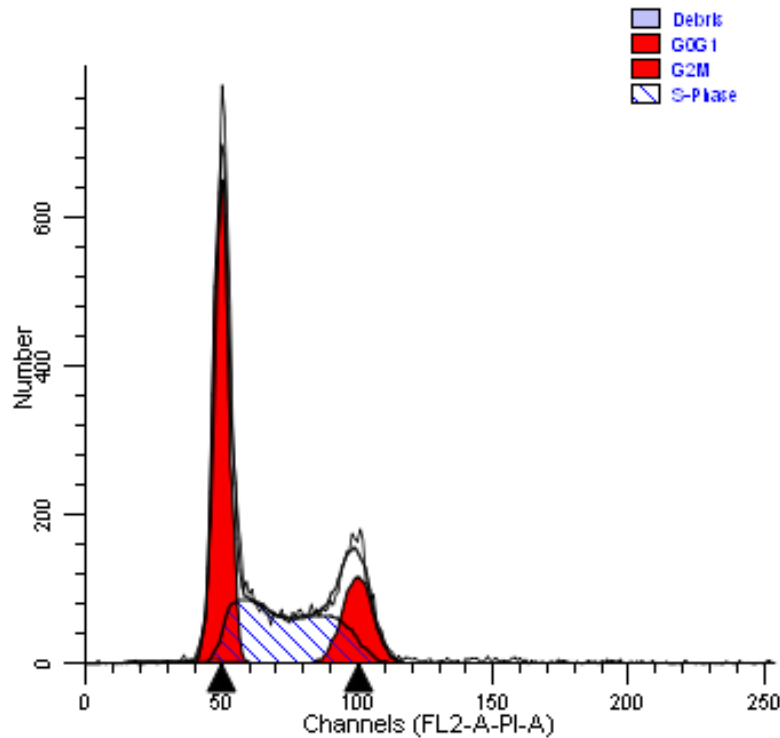
Modeled Events: 12971

RCS: 16.161

You can fix this by turning off the auto-aggregates and making your doublet-discriminator gate tight around the single cells.



By doing this, it brings the RCS value down to 2.601.



Sync Wizard Model

File: MK_030811.001

Date acquired: 08-Mar-11

Date analyzed: 23-Mar-2011

G0G1:

46.95 % Mean: 50.21

CV: 5.64 %

G2M:

16.84 % Mean: 100.42

G2/G1: 2.00

S-Phase:

36.21 % Mean: 74.03

Compartment 1: 14.65 %

Compartment 2: 10.21 %

Compartment 3: 11.35 %

Debris: 1.88 %

Modeled Events: 10107

RCS: 2.601

4. When going through the subsequent files in your batch analysis you can edit the batch properties to 'Fit with current model' so you do not have to click analyze every time. You can also uncheck the Database and File prompt so when you switch through the files you are not prompted to choose the parameter and gates every time.

Edit Properties for File Processor

Name	Value	Description
Analysis	Fit with current model	Analysis option when file is opened.
Save report	<input type="checkbox"/> Off	Auto-save report?
Save report path	C:\Documents and Settings\Administrator\My Docu	Folder in which to auto-save reports.
Print report	<input type="checkbox"/> Off	Print report?
Save graphics	No	Save graphic files.
Prompts		
Database prompt	<input type="checkbox"/> Off	Prompt to confirm save record?
File prompt	<input type="checkbox"/> Off	Prompt to confirm gates or histogram selection?
Multi-dataset prompt	<input type="checkbox"/> Off	Prompt to choose a dataset from multi-dataset FCS files?

OK
Cancel
Reset
Help...