External clock for synchronization

Dear Justin,

I opened the FBD file you sent. Obviously the frequency is out by exactly a factor of 2. I read it with a frequency of 10,000,000 for the FLIMbox frequency and the files reads fine. Below is the image I get.

The logic should be the following

When the 3 axis card uses internal clock (it should be the default) the frequency should be 10,000,000. When the 3 axis card is in external clock (Used only for scanning FCS or to synchronize with the Ti:Sapphire laser, see below) then the frequency should be 20,000,000. In the screen you sent me you have ext clock for the 3 axis card and 10,000,000. It should have been 20,000,000. This explains the problem with the image. However, the FBD file is the raw data and it can be reprocessed correctly as shown in the figure above.
Below is the logic for using the external clock

Imaging only: For normal imaging, the 3-axis card can be set to internal clock (10,000,000), the ISS-FCS card can also be in internal clock and the same for the FLIMbox. If you are using version 0.2 for the ISS-FCS card the internal frequency is 50MHZ and if you have version 1.1 the internal clock is 24MHZ. If you are using the FLIMbox, the clock of the FLIMbox as declared in the FLIMbox image page should be 20MHZ. The reason why you can avoid synchronization and use just the internal clock for imaging in every case is because SimFCS resets the start of image after each frame. So there is no carryover of the slight mismatch of the 3-axis card internal clock and of the acquisition card, the frame is always OK. This is the default mode. If the system file is re-initialized or a new user sign for the first time, SimFCS uses these parameters.

More on imaging only if used for scanning FCS: If you want to do scanning FCS, then there is no possibility to reset after each frame, since there is no frame. Therefore, for a long acquisition, there is a slight shift of the clocks of the 3-axis card and of the acquisition card, either ISS-FCS or FLIMbox. Therefore, in this case the clocks of the two cards need to be synchronized.

For the ISS-FCS card this is done using the clock of the tower, which puts out 20 MHZ (according to Jeff). This clock must be sent to both the ISS-FCS card and the 3-axis card. Then you must set both of these cards to use external clock and the frequency must be 20 MHz for both. Furthermore, to start the data acquisition exactly on the same clock cycle, you must set the “use trigger for cards” flag in the system parameter page of SimFCS. This mode requires the “trigger” connection, which is done using the new cable that Jeff sent to you.

For the FLIMbox, it is the FLIMbox that generates the 20 MHZ clock. This is done using a connector in the FLIMbox unit labeled “synch out”. Since this modification was done about 1 year ago, it could be that your unit lacks of this connector. Therefore, apparently your system could not be synchronized for scanning FCS, although it is in synch for imaging. However, the old model of the FLIMbox always sent out a signal at 20 MHZ except in internal clock. When in external clock you have 2 firmware’s, one to be used with the Ti:Sapphire laser and the other to be used with the ISS lasers that are modulated at 20 MHz. If you are using the 80MHz laser, then this laser frequency is applied to the FLIMbox input, it gets divided by 4 to give 20 MHz. The 20 MHZ is available at the frequency out of the FLIMbox. This 20 MHZ signal can be used to synch the 3 axis card. If you are using the EXT/INT firmware of the FLIMBOX the output is also at 20 MHZ. This output must be split and one part sent to the 3-axis card and the other either to the diode for modulation or to the FLIMbox input to close the loop. Therefore, even if you don’t have the latest FLIMbox hardware version, you should be able to do all the functions needed for scanning FCS.

The only difference between the various forms of operation is that under one form, you should never exchange cables. That is if you are “always” using the FLIMbox, then once the cables have been connected you should not need to change them. If you are always using the ISS-FCS card, then use the combination for the ISS-FCS card. If you want to switch from one card to another and you don’t have the newest hardware version for the FLIMbox and you are using the Ti:Sapphire and you want to be able to do scanning FCS, then you need to switch cables. With the instructions I am giving you in this report it should be easy.

Ciao