The FLIMbox as of November 11, 2008

During the past weeks, with Enrico D’Amico we have made small but significant changes in the operation of the FLIMbox. The motivation for the changes was to provide one more input to the FLIMbox to synchronize with external source, mainly the scanner and its operations. In the original Ryan design, the scan enable line was simply turning on (or off) the entire FLIMbox. In the new design, the scan enable line is registered along the data stream and the software decides when the data correspond to valid data, such as during frame acquisition. This new mode of data acquisition has allowed us to use the FLIMbox for single point FCS (when a scan enable signal is not available) and for circular scan. The circular scanning with the ISS instrument required an additional change in which the clock of the 3-axis card was synchronized with the clock of the FLIMbox.

During this development, we noticed that some of the designs were working and some others failed. After reviewing all the circuits, we realized that most of the problems were with some critical high frequency internal operation of the FLIMbox that became unstable, giving an erratic behavior. In fact, some of the boards were working with a given design and some others were not. The critical value is reached when the internal operation frequency of the FPGA reaches the value of 190MHz or above. This value is obtained when the FPGA generates 4 windows at 48 MHZ (4*48MHz=192MHZ). The maximum reliable operating frequency for all our boards is just too close to the critical value. This prompted us to change the internal design so that this limit is never reached.

One critical change was to modulate the light at high frequency (For example 80 MHz or 40 MHZ) but to run the FPGA at half that frequency. For example, with the 2-photon laser (at 80 MHZ) the laser signal to the FLIMbox is first divided by 2 so that the internal operation of the FPGA is now 40 MHZ. When using 4 windows, the maximum internal operating frequency is 4*40MHz=160 MHz, well within the limit of operation of the FLIMbox. This division by 2 of the external synchronization fixed most of the problem, however, added some limitations to the operation of the FLIMbox. These limitations are hardly seen in the normal phasor analysis. However, if we want to exploit the multi harmonic capabilities of the digital frequency-domain approach for example using the Fianium white laser, we will not be able to operate the system at very high frequency. The major problem with the 4 windows design is the 4th harmonic is multiplied by zero so that we have no modulation at this frequency. When dividing the clock by 2, to safely operate the FLIMbox, the following table gives the available harmonics.

<table>
<thead>
<tr>
<th>Laser modulation</th>
<th>Fundamental and harmonic available</th>
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</thead>
<tbody>
<tr>
<td>80 MHz (4w, base at 40 MHz)</td>
<td>80 MHz, nothing at 160 MHZ and weak harmonic at 240 MHz</td>
</tr>
<tr>
<td>40 MHz (4w, base at 20 MHz)</td>
<td>40 MHz, nothing at 80 MHz, 120MHZ, 200MHZ,</td>
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</table>

Although the card works in a stable mode, the lack of true second harmonic of the laser can be problematic.

For this reason, we explored a different design that operates with 8 windows, but at smaller than 24 MHZ) laser modulation frequency. We realized one version operating at 20 MHz which gives the following set of frequencies.
20 MHZ (8w, 1 channel)

20 MHz, 40 MHz, 60 MHz, 80 MHz, 100 MHz, 120 MHz, 140 MHz, nothing at 160 MHz, 180 MHz, 200 MHz, 220 MHz

As you can see, the only missing harmonics is the 160 MHZ, but the 140MHz and the 180 MHZ are available. A problem with this design is that it has only one channel. We are working now to produce a firmware version that will have 2 channels or we could use 2 boards.

After this introduction, we can now better understand the new firmware and the various checkboxes in the FLIMbox page of SimFCS.

The stable firmware that divide the clock by 2 and have the synch signal for the 3-axis card are the following

- FLIMhet_0_6_2_2ndHarmonic_SynchOut.fbf (to use for 48MHz modulation on the second harmonics)
- FLIMhet_0_6_2_IntClk_SynchOut.fbf (to use for single point FCS when lifetime is not needed)
- Flimhet_0_6_2_80MHz_2ndHarmonic.fbf (to use with the 2-photon laser)
- FLIMhet_0_6_5_8w_1ch.fbf (to use for one channel, 20 MHZ laser modulation, multi harmonic mode)

80 MHz laser, 4 windows, using the second harmonics. The firmware works with 4 windows, using a divide by 2 for the laser. In this way the card works at 40 MHz internally. Check second harmonic and the Digilent decoder.

48 MHz operation. There is no stable working firmware for 4 windows and 48 MHz. The only system working at this speed is that FLIMbox we have on the Zeiss system. All the other boards we tested have instabilities. To operate with the Olympus and the ISS-Alba, we use the 2\textsuperscript{nd} harmonic firmware with the Digilent decoder.

20 MHz and 8 windows 1 channel. This firmware is in rapid change as we are generating a version that will operate with 2 channels.