Using a Prairie Technologies laser-scanning microscope system with ScanImage 3.8.

Version 1 (11/4/2012)
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I'm happy to clarify things, but I obviously take no responsibility over the health of your equipment.

I've merely compiled my notes on getting our new system up and running, Nathan Wilson (MIT) pointed out the key features of the Prairie galvo control box to me when we set up our older system and deserves any thanks for this. We have had basic ScanImage functionality working on an older install (~2007) and now a new system (2012) and the general ideas should apply to any of their installs. If you follow this you will be able to run ScanImage 3.8 with your Prairie system, but you will still need your Prairie computer to control the voltage on the PMTs and the pockels. I highly recommend you set up pockels control in ScanImage, which is easy to do, but will require another NI-DAQ board with analog output, I explain how to do this at the end in the *optional* section.

We have a resonant galvo as well, and I am investigating how to use it with ScanImage 4.1, but it looks like it will be tricky and involve the purchase of Thor Labs' galvo driver box because ScanImage 4.X relies on the Thor Labs API to talk to the resonant scanner and it's DAQ board. I will update this if I get that working.

Items You Need:
Basically all the requirements for any ScanImage install apply. The key item is a fast National Instruments DAQ board with high input range (either the PCI-6110, 6111 or 6115, which is the same board Prairie installs with its systems to drive the galvos at the high rates needed. Please consult the ScanImage documentation for hardware requirements.

I recommend a wire to BNC adaptor like this one:
http://www.digikey.com/product-detail/en/1296/501-1033-ND/603342
So that you can easily route a trigger signal (explained later).

You will also need some non-braided jumper wire, a few spare BNC cables and BNC t-splitters http://www.digikey.com/product-detail/en/5-1634532-1/A97571-ND/1755958

Directions
Assuming you already have a computer, with a fast NI board installed as well as the correct version of DAQmx that ScanImage supports (9.3 is the version I used). Connect the output of the card to a breakout board like the NI-BNC-2110 and set that aside.

<u>Gavlo Controls</u>
Locate the Prairie galvo control box and look for the X2 and Y2 inputs as well as the "Switch" connection (see picture 1). You can **connect a**

1. Back of the Prairie galvo control box. The X2&Y2 inputs allow for auxiliary control if the voltage is high (TTL; 5V) on the "Switch" connection.

BNC cable from the X2 and Y2 inputs to your NI Board's analog out channels (AO0 for X2 and AO1 for Y2). This will allow you to give galvo commands from ScanImage via the PCI-611X board. You have to toggle the "Switch" input with a TTL input (5V) to tell the box ScanImage will be controlling the galvos. To do this you can route one of the digital outputs of the NI board to this "Switch" just like you would do for a shutter. **Connect a BNC to "Switch."** You can route the X and Y Feedback connections anywhere you like if you want to record the galvo movements, but don't waste the analog inputs on the PCI-611X board for this, you need that speed for the PMT signal.

PMT Signals

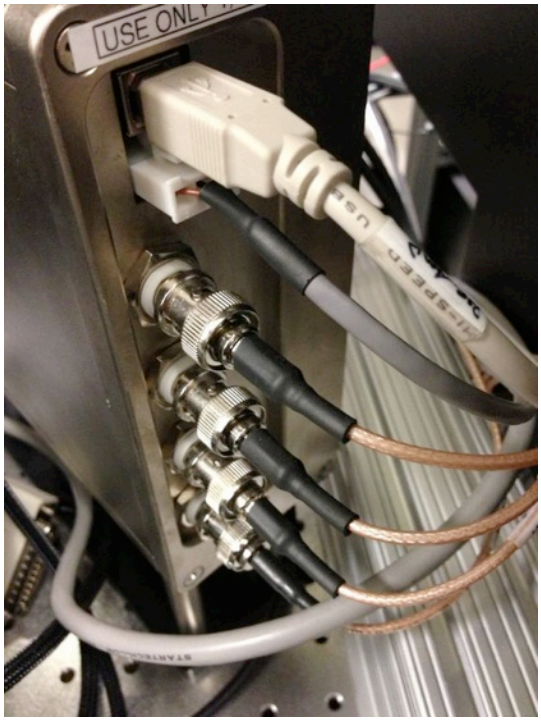
Prairie systems vary in how PMT signals are dealt with. In our system the PMTs are driven by a Prairie power supply and the signals from the PMTs are routed into a pre-amp and then passed to a PCIe board in the Prairie computer that I am unfamiliar with. In the older system I am pretty certain they were routed into the breakout box for the PCI-611X, likely with a preamp in between. **You need to t-off the signal into the ScanImage board, but were you do this will vary.** Please be a bit careful here and make sure you don't accidentally pass a high voltage control signal into the PCI board. The PCI-611X has a higher input voltage range than R, M and X series boards that are often used for patch-clamping etc., but will fry if you pass voltages in excess of 42 V. **Picture 2**, on the right, shows the silver preamp that we have installed.
The BNCs connected on the front are taking the raw PMT signal, you want to t-off the signal from the back.

Picture 3 shows the **back of the preamp, this is were you can install t-junctions for the PMT channels you want to connect into your ScanImage board**. For us these cables were ordered from deep red to blue. I've tested things with one channel t'd off and did not note any obvious interference in either direction. It should be noted that splitting the signal can under particular conditions lead to poor results (cross-talk, ground-loop issues, etc.). If you have problems from splitting I would suggest just switching the BNC cables (ScanImage vs. Prairie) each time you use a particular setup.
Regardless of which you choose, you should now have a BNC cable destined for your ScanImage box from your PMTs, Gavlo X2, Galvo Y2 and the Gavlo "Switch".

Shutter Control

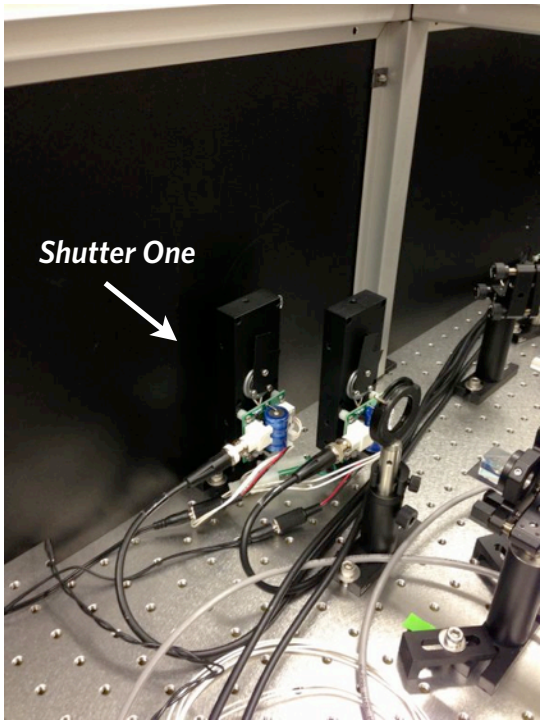
Prairie installs a "hard shutter" after the pockels cell just before entering the scan path (**Picture 4**). ScanImage

2. The Prairie PMT preamp. **The inputs on the front are from the PMTs and should be left in place.**

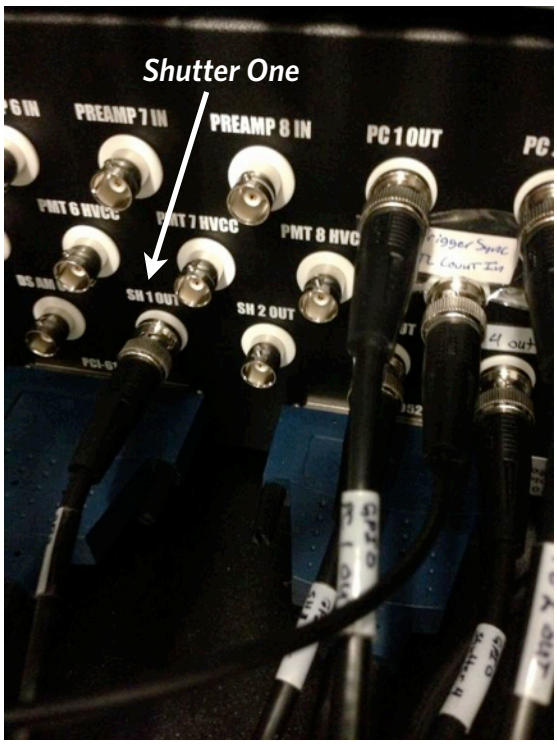


3. The back of the Prairie PMT preamp. **The outputs are all BNCs (ignore the usb and molex connectors) and are ordered by color. These are the signals to route to ScanImage.**

knows how to deal with this kind of shutter and will open it when appropriate using one of the digital outs on the PCI-611X board. To take advantage of this you will need to route a BNC cable from the Prairie "GPIO" box to the ScanImage board. You may have multiple lasers setup. It is common to have an "imaging" laser and an "uncaging" laser. For our purposes here we are only concerned with the imaging laser. Find the shutter that is front of your imaging laser's optical path (with the laser off of course; **Picture 4**) and trace it's input to the Prairie GPIO box. Picture 5 shows the GPIO connector from where our shutter's input comes from. Our's was labeled SH 1 OUT, I would not be surprised if yours was labeled the same. Y**ou will need the ScanImage board to give a TTL input to the connected BNC (that came from the shutter)** and conceptually you could use a splitter on the GPIO box and connect the BNC already there to one side and a new BNC that you will connect to the ScanImage board on the other. If you do this, you should keep in mind that when you give a shutter command from Prairie it will travel to the digital I/O pin on your ScanImage board and likewise a shutter command from ScanImage will flow to the shutter and to the GPIO box. This shouldn't hurt anything and I've tested it to make sure it works, but if your concerned about errant voltages routing to unintended boards, I suggest building a switch of some sort or swapping cables.



4. Prairie's shutter. Our imaging laser's shutter is labeled "Shutter One." You will need to trace the path of your imaging shutter's BNC input to the GPIO box (see next picture).



5. The Location of the Shutter Output. You will need to give 5V over this BNC (connected to SH 1 Out), you can use a splitter here, but see text for warning.

<u>Final Board Arrangement & Triggering</u>

At this point you should have BNC cables connected to the PMT signals you want, X2 galvo, Y2 galvo, galvo "Switch" and the laser shutter. You will want to connect these to your NI-BNC-2110 or your NI-2090 (rack mount block). In addition, you will need to connect the right jumpers on the digital I/O pin section. I suggest the following for wiring your board:

X2 Galvo -> Analog Out 0 (AO0)
Y2 Galvo -> Analog Out 1 (AO1)
PMT 1 -> Analog In 0 (AI0)
PMT2 -> Analog In 1 (AI1) etc.
Galvo "Switch" -> User 1
Laser Shutter -> User 2

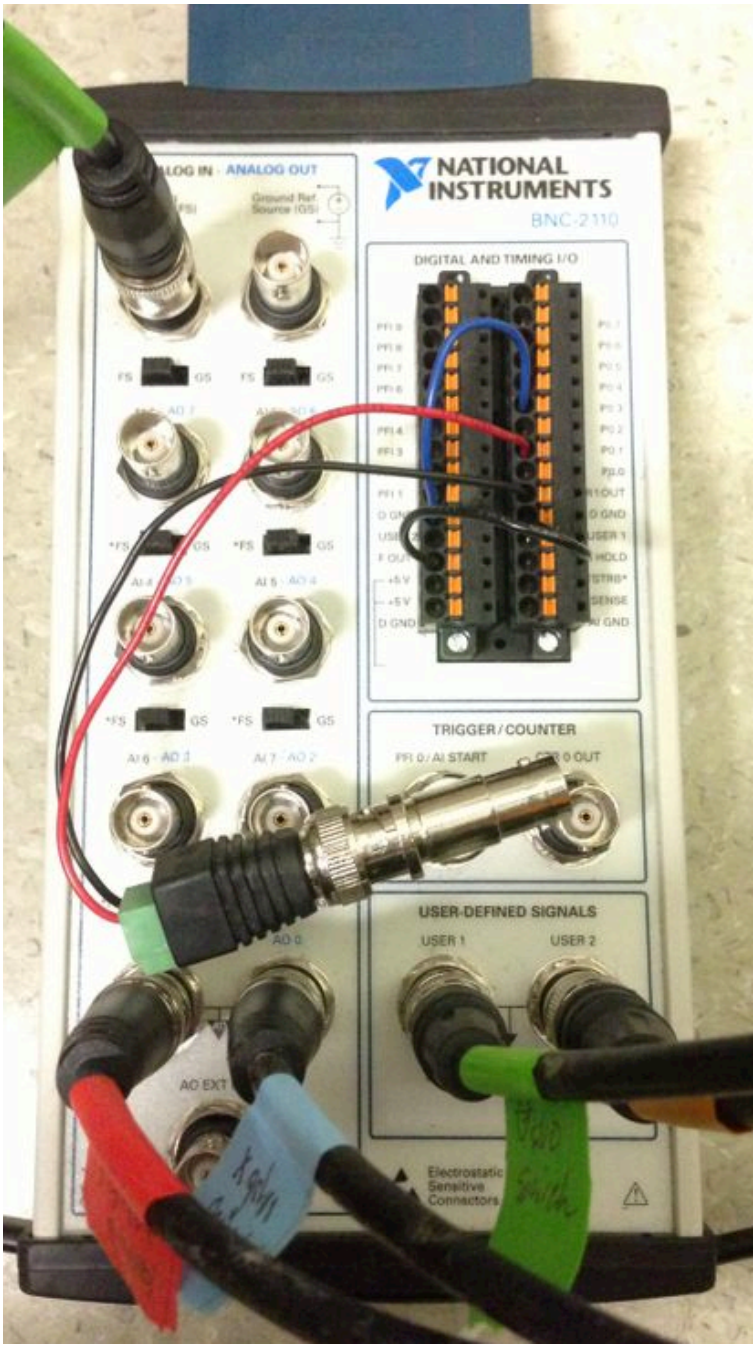
We will then connect the digital jumpers in the following manner:

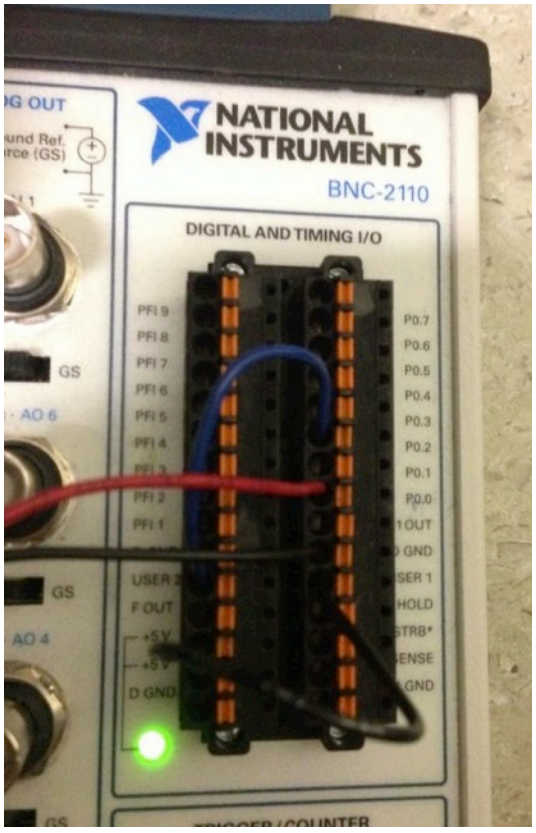
Bridge P0.2 to User 2
Bridge a +5V hole to User 1

The digital I/O pin section of the BNC-2110 or 2090 may be a bit of a mystery to you, if so, the only trick is to take a small flat head screw driver and depress the orange tabs,

which will open up a clamp that once you let go will crimp a wire you put in there. The "User 1" and "User 2" are setup for you to be able to route a particular digital pin to it and then subsequently interact with those pins over BNC connections labeled "User 1" and "User 2" also on the board. See **Picture 6** for how I routed our digital pins.

You will also need to set up a trigger, that ScanImage will use to synchronize its input/output. To do this you will need to connect the digital P0.0 pin on your board and ground on the board to a BNC connector. Connect the BNC connector (now with the P0.0 and ground wires attached) to the PFI0 BNC terminal on the board (see





6. (above) Digital I/O organization of the ScanImage board. The blue wire routes the shutter output, which flows through P0.2, to User 2. The red and black pair, below the blue, route ground and P0.0 to a BNC connector (not shown) that is plugged into PFI0 (also not shown) to route a trigger signal. The second black wire routes the +5V pin on the left to User 1 on the right.

7. (left) Final board organization. The ScanImage trigger is routed from P0.0 to PFI0, and a splitter is put in place to allow routing the trigger to another board (*optional*; not shown). The upper left green labeled BNC carries the PMT signal into AI0. The galvo "Switch" BNC is placed into User 1's BNC connection. The shutter signal is passed over User 2, the other end is connected to the BNC that Prairie installed on the shutter at the back of the GPIO box. The X2 galvo control is in the lower left (blue tape) and is connected on A00. The Y2 galvo control (red tape) is connected on A01. User 1 (Galvo Switch) can be disconnected to give back control of the galvos to Prairie View.

Picture 7). You can install a splitter on PFI0 if you want to also route the ScanImage trigger to other boards (for controlling stimulators etc.).

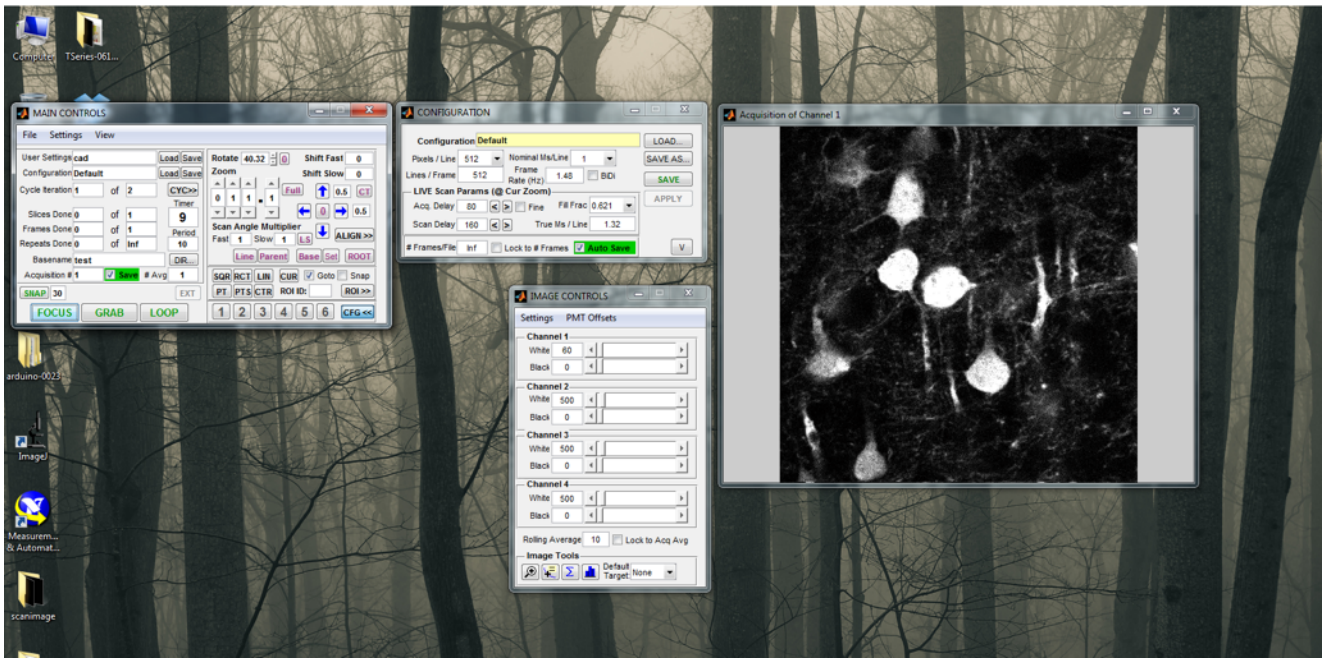
ScanImage Configuration

You will need to modify the model ScanImage configuration file. This file is located in the ScanImage directory the path of which is: /SCANIMAGE_r3.8/ScanImage/init_files/standard_model.ini
Open up standard_model.ini in a text editor or in Matlab.

First, you will need to know the name that DAQmx (NIDAQ driver) gave to your board. It should be 'Dev1' or 'Dev2' etc. If you do not know this open up the national instruments program on your desktop (blue N) and look under the devices tab and your PCI-611X board will be listed as "DevX" where X is the number it chose. I'll assume it's Dev1 for now, but just change any mention of Dev1 to DevYourDevice if you have something different.

The first two keys to modify are in the init structure: 'acquisitionBoardID' and mirrorOutputBoardID should both be 'Dev1'. Under that you will see 'XMirrorChannelID' and 'YMirrorChannelID' these should be set to 0 and 1, respectively. Scan angular range should be left alone unless your galvo situation deems otherwise. The tricky part comes when setting 'voltsPerOpticalDegree' because chances are Prairie didn't tell you what galvo set they sold you. However, chances are you have a Cambridge Technology 6210/6215 and unless you asked for it, or it is in your quote, you likely have 3mm mirrors and not 6mm. If you asked for larger galvos to help fill larger objectives like the popular Olympus 20x 1.0 NA then you have the 6mm version. Prairie will tell you what you have if you ask nice, and you will have better luck talking to the tech who installed your system. You can probably open up the control box to know for sure, but there is a 99% chance you have the 6210/6215 either 3mm or 6mm. For 3mm enter the recommended 0.333 value and for 6mm enter 0.5. I don't recommend pulling the galvos out of your scanhead (the Ultima) or you will mess with the alignment.

Set the 'triggerBoardID' to 'Dev1', the 'triggerLineID' to 0 (for P0.0) and the 'triggerInputTerminal' should be set to 'PFI0'. You can configure pockels control (optional, not documented) if you so choose in the 'Beam' section.



8. Using ScanImage with a Prairie system.

In the "Shutter Configuration" section set 'shutterOn' to 1, 'shutterBoardID' to 'Dev1', 'shutterLineIndex' to [2] (for P0.2), 'open' to 1 and 'shutterOpenTime' can be left at 0, but feel free to change this if needed. Save your modified file somewhere handy. You should now be ready to collect images in ScanImage, although you will need to control the PMT voltages in Prairie View.

Optional

If you would like to do more with your setup like control your pockels or give stimuli you will need another NIDAQ board. You will not need an expensive PCI-611X board. I would recommend an X-series board like the PCIe-6343 or even better the PCIe-6353. You may have an X-series board around your lab or their predecessors the M and R-series, these are all fine. You will need a break out board like the BNC-2110 for this board too. I'll assume you have one of these boards, it's installed in your machine and it's called 'Dev2'.

Pockels

Prairie sold you standard Conoptics pockels cells. The control boxes for these are pretty standard and take an optional control input on the back (Picture 9). The control takes a signal between 0 and ~2 volts, but you won't get much more power above 1.5V. In the 'Beam' section of the ScanImage configuration you need to tell it the control board for your pockels is Dev2 and tell it the analog output channel (AO0 is a good choice) for your control signal, I would leave the default voltage max to 1.5V or change it if you know you want a different peak voltage. Place a splitter on the back of the conoptics box to use both the Praire BNC and ScanImage BNC in tandem (remember my warning about passthrough of errant voltage signals), or switch the cables out at will.

9. Back of the conoptics pockels cell control box. There are a variety of options for controlling the pockels, ScanImage and Prairie View use the same scheme so you can safely leave everything as is and just swap out Prairie's BNC with yours, or add a splitter.

Stimuli

You can make Dev2 a slave to your ScanImage board by connecting the PFI0 terminal on the ScanImage board (remember the splitter there) to the PFI0 terminal on Dev2. This will allow you to send voltage commands from it on the other analog outputs, relative to the start of image acquisition.