



Ultima Multiphoton Microscopy System User Manual version 2.5

PrairieView TriggerSync

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Table of Contents

1	Preface	8
2	Safety Precautions.....	9
2.1	Warning and caution symbols used in this manual	9
2.2	Warning labels used on the Ultima Multiphoton Microscopy System....	13
3	Introduction	14
3.1	Scope of this Manual	14
3.2	Conventions Used in This Manual	14
3.3	Ultima Overview	14
4	Hardware and Electronics.....	16
4.1	Overview.....	16
4.2	Rack and Other Electronic Components.....	17
4.2.1	PC	17
4.2.2	PMT dual preamplifier & HV control unit.....	17
4.2.3	National Instruments BNC breakout boxes & boards	18
4.2.3.1	NI 6052E board	18
4.2.3.2	NI 6713 board.....	19
4.2.3.3	NI 6110/6115 Board – pixel data sampling methodology	19
4.2.3.4	NI BNC-2090 & BNC-2110 boxes	19
4.2.4	Software communication through NI hardware.....	23
4.2.5	Device Control Box and DCRI	23
4.2.6	Prairie Preamplifier	25
4.2.7	Galvos & Acousto-Optic Deflector (AOD)	25
4.3	Optics	27
4.4	Laser Light Path	27
4.5	Ultima Scan Head.....	28
4.5.1	Dichroics.....	28
4.5.2	Microscope	29
4.5.3	Detectors	29
4.5.3.1	Top-Mounted detectors	30
4.5.3.2	Sub-Stage detectors.....	30
4.5.3.3	Dodt Gradient Contrast detector.....	31
5	Ultima Startup Procedure	32
6	Alignment and Calibration Procedures	33
6.1	Beam Alignment	33
6.1.1	Imaging Beam Alignment in Scan Head	33
6.1.2	Uncaging Beam Alignment in Scan Head.....	34
6.2	Spot Detector Alignment.....	35
6.3	Calibrate Point Image	36
6.4	Resetting the PMT High Voltage	36
7	<i>PrairieView</i> Software	37
7.1	<i>PrairieView</i> - Main Window.....	37
7.2	Main Window Controls.....	38

7.2.1	Scan Resolution Tools.....	39
7.2.1.1	Image Size	39
7.2.1.2	Pixels Per Line & Lines Per Frame.....	39
7.2.2	Zoom and Dwell time Tools	40
7.2.2.1	Dwell Time Per Pixel	40
7.2.2.2	Optical Zoom	40
7.2.2.3	Window Size.....	40
7.2.3	Scanning & Image Window Tools.....	41
7.2.3.1	Live Scan/Stop Scan	41
7.2.3.2	Single Scan	41
7.2.3.3	New Window	41
7.2.3.4	Average/Sum.....	41
7.2.4	Shutter and Imaging Mode	41
7.2.4.1	Hard Shutter Closed/Open	41
7.2.4.2	Soft Shutter Closed/Open	42
7.2.4.3	Scan Mode: Galvo or AOD	42
7.2.4.4	Label and Objective Lens Selection	42
7.2.4.5	Label Selection.....	42
7.2.4.6	Objective Lens.....	42
7.2.5	Pan and Scan Rotation Tools	43
7.2.5.1	Pan Control and XY Stage Control.....	43
7.2.5.2	Scan Rotation.....	43
7.2.5.3	XY Stage Control.....	44
7.2.6	Laser, PMT, DAQ	44
7.2.6.1	Laser	44
7.2.6.2	PMT Gain (voltage)	44
7.2.6.3	DAQ Gain	45
7.2.7	2P Laser	45
7.2.8	Z-series	45
7.2.8.1	Z-Motor Control	46
7.2.8.2	Z-series Calculator	46
7.2.8.3	Collecting a Z-series over a large depth	47
7.2.9	T-series	49
7.2.9.1	T-series definitions	49
7.2.9.2	Collect a Basic T-series.....	50
7.2.9.3	Collect a Complex T-series	51
7.2.10	XY Stage	52
7.2.10.1	XY-Stage definitions	52
7.2.11	Misc.....	53
7.2.12	Scan Information	54
7.3	PrairieView Toolbar Options	55
7.3.1	File.....	55
7.3.2	Tools.....	56
7.3.2.1	Calibrate Objective Lens	56
7.3.3	Window.....	57

7.3.4	Display.....	57
7.3.5	Help	57
7.4	Image Window	58
7.4.1	Selecting an Input Channel (Ch1/Ch2/Ch3/Ch4).....	60
7.4.2	'BOT' – Brightness Over Time	61
7.4.3	'LS' – Line Scan	62
7.4.4	'LUT' – Look-Up Table.....	65
7.4.5	'ROI' – Region of Interest	67
7.4.6	Snap Tool	68
7.4.7	'PA' – Photoactivation.....	69
7.4.8	' μ m' – Line Profile Measurement	70
7.4.9	'MIP' - Maximum Intensity Projection.....	71
7.4.10	'MP' – Marked Points.....	72
8	TriggerSync Software	74
8.1	Main Operational Modes.....	74
8.1.1	Analyze Acquired Data: Off	75
8.1.2	Analyze Acquired Data: On	76
8.1.2.1	View Channel Sensitivity	76
8.1.2.2	'Change Scale' and 'Auto Scale'	76
8.1.2.3	'Sequential File'	77
8.1.2.4	'Save Experiment'	77
8.2	Acquisition and DAC Output Setup.....	78
8.2.1	Acquisition Setup.....	79
8.2.2	Stimulus Control	80
8.2.3	Protocols	82
8.2.3.1	I/V (Current / Voltage)	82
8.2.3.2	Ramp.....	82
8.2.3.3	Pulse/Train	83
8.2.3.4	G.F.C. (General Fluorescence Control).....	83
8.2.3.5	Custom Waveform.....	83
8.2.4	Use Marked Points During Acquisition	84
8.3	ADC Input Controls.....	86
8.3.1	Selecting Input Channels.....	86
8.3.2	ADC Configuration.....	87
8.4	Point Calibration	89
8.4.1	Overview	89
8.4.2	Calibration Process	89
8.4.2.1	Manual Calibration	97
8.4.2.2	Automatic Calibration	97
8.5	Marking Points.....	98
8.5.1	Calibration Files and General Controls.....	99
8.5.2	Marking points – Basic Controls	101
8.5.3	Line Definition.....	103
8.5.4	Mapping Setup	104
8.5.5	Functional Map Display	109

8.5.6	Miscellaneous Controls	111
8.5.7	Use Marked Points During Acquisition	112
9	Acquisition Scenarios	114
9.1	TriggerSync Electrophysiology Acquisition	115
9.2	Line scans & Electrical recording.....	116
9.2.1	Line scanning with electrical recording & stimulation.....	116
9.2.2	Viewing Line Scan data	116
9.3	Electrical recording & Uncaging.....	118
9.4	Line scans, Electrical recording, & Uncaging.....	119
9.5	Non-Line Scan Images, Electrical Recording & Uncaging	120
9.6	Functional Map Acquisition.....	122
9.6.1	Display and analysis of Functional Maps.....	122
	List of Figures	123
	List of Tables	125

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1 Preface

Every effort has been to ensure that the data given in this document is accurate. The information, figures, tables, specifications, and schematics contained herein are subject to change without notice. Prairie Technologies, Inc. makes no warranty or representation, either expressed or implied, with respect to this document. In no event will Prairie Technologies, Inc. be liable for any direct, indirect, special, incidental, or consequential damages resulting from any defects in its documentation.

2 Safety Precautions

2.1 Warning and caution symbols used in this manual

The Ultima Multiphoton Microscopy system is designed with the utmost safety of the user in mind. However, improper use or failure to follow safety instructions may result in personal injury and or property damage. Please read this manual before operation to ensure proper use of the system.

Safety instructions in this manual are accompanied by the following symbols to highlight their significance. Please pay attention to the instructions highlighted by these symbols:



Disregarding the instructions accompanying this warning symbol may lead to serious bodily injury or death.



Disregarding the instructions accompanying this warning symbol may lead to serious bodily injury or instrument damage.



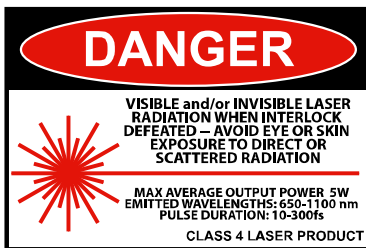
WARNING

Use of this systems, components, or performance of procedures other than that specified in this manual may result in hazardous radiation exposure.

Intended use: *This system is designed as a Class 1 laser product, intended for use in laser-based imaging microscopy. It is not intended for any other purpose.*



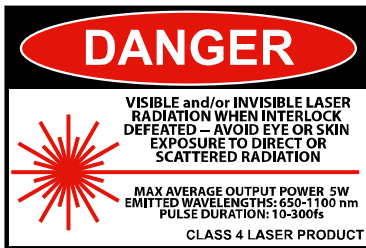
WARNING



Interlocked safety covers: *This system is enclosed within a light-tight and laser-safe covers. These covers are designed to protect the user from exposure to Class IV laser radiation. Therefore, at no time should these covers be removed or modified. The interlocks on these covers are designed to be defeated by authorized factory-trained personnel during specific maintenance and service procedures. During these procedures, appropriate laser-safe eyewear is required.*



WARNING



Never look into the laser beam path! The laser used in the Ultima system is a Class 4 infrared (IR) laser. It does not have a beam visible to the naked eye. The optics used in this system may also cause some back-scattered or reflected laser light. It is therefore critical to operate this system following all safety instructions and wearing appropriate laser-safe eyewear.



CAUTION

A. Installation of the Ultima Multiphoton Microscopy System: To ensure proper installation of this system, it must be installed by Prairie Technologies technicians.

B. Do not disassemble: Disassembly of this system may result in electrical shock and other hazards., including exposure to Class 4 laser radiation.

C. Power supply cords: The Ultima system comes with all necessary power cord. Do not change or replace them as use of an improperly rated power cord may result in system malfunction or failure.

D. Prevent contact with moisture: Moisture contact with any component of the system may result in a short-circuit or damage to optical components. If water gets into a system component, immediately discontinue use of the system, turn off power, and contact Prairie Technologies immediately.

E. Handle with care! This system is designed as a precision optical instrument. Each optical and electronic component has been placed with great care to assure optimal system performance. Do not pull on or bend cables or fibers. Do not handle filter cubes or dichroics except as recommended by the manufacturer.

2.2 Warning labels used on the Ultima Multiphoton Microscopy System



Warning label on beam cover and light box



Warning label on interlocks components



Warning label for defeated interlocks (on interlock defeat bocks)



Warning label for defeated interlocks (on interlock defeat bocks)



Laser Warning-IEC Logo

3 Introduction

3.1 Scope of this Manual


Congratulations on your purchase of a Prairie Technologies Ultima Multiphoton Microscopy system. This manual is intended to provide the information necessary to operate your Ultima system and describes not only the hardware but also the *PrairieView* software for image collection and scanner control and the *TriggerSync* software for synchronizing imaging with electrophysiological or uncaging events.

3.2 Conventions Used in This Manual

When referring to a specific button or icon, single quotes (‘ ’) are used:

Press the ‘**Single Scan**’ button to collect an image.

When describing a specific action that the user is to take, particularly as a part of a sequence of actions, an arrow is used to highlight the action:

- Press 
- Click and release the mouse once at one corner location of the ROI.
- ...

Hyperlinks are present throughout the manual. Each entry in the Table of Contents is a link. Internal references to other sections are also links and are indicated with [blue text](#).

3.3 Ultima Overview

The Ultima is a unique laser scanning microscopy instrument that is capable of using one or two laser beams for multiphoton imaging and/or uncaging experiments on tissue slices or very small animals. With only one laser, it is possible to perform traditional raster-scanned laser imaging and (optionally) perform sequential imaging and uncaging experiments. When two lasers are used, it is possible to image and uncage simultaneously. In either scenario the operator can choose to deliver electrical stimuli to the specimen as well as recording electrophysiological signals from the specimen through the use of trigger-syncing software.

The Ultima IV is a version of the instrument designed specifically for *in vivo* studies of small to medium sized animals. It is available in two versions, one in

which the optics are fixed and the other which allows the optics to translate in the X & Y directions.

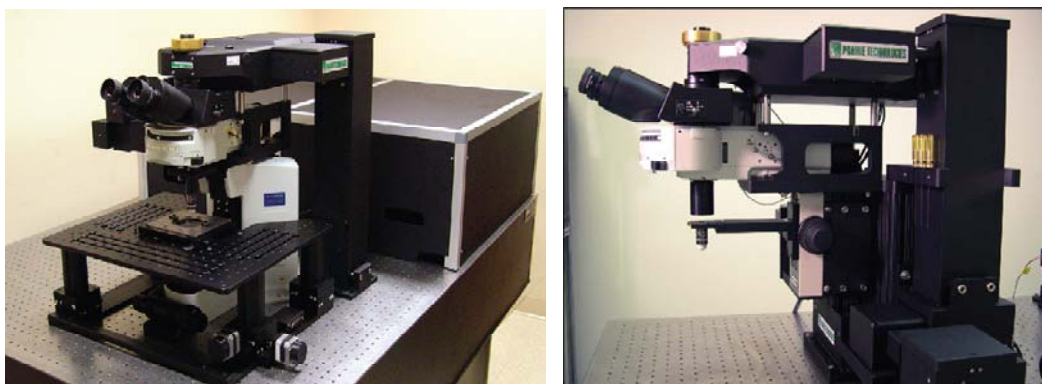


Figure 3.1: Ultima (left) and Ultima IV (*in vivo*) system (right) without light boxes

The Ultima uses externally mounted photomultiplier tubes (PMTs) to collect the fluorescence emitted from the specimen. These detectors are located adjacent to the left of the epi-fluorescence illuminator of the microscope. Optional sub-stage detectors may also be present, located to the left of the microscope base below the stage.)



CAUTION

PMTs are extremely sensitive to light. Care should be taken to eliminate all sources of stray light from reaching the PMTs, as this will dramatically reduce the sensitivity of the system. Prairie Technologies provides a light-tight and laser-safe enclosure around the instrument to ensure optimal performance as well as to provide a safe working environment for the users of this instrument.

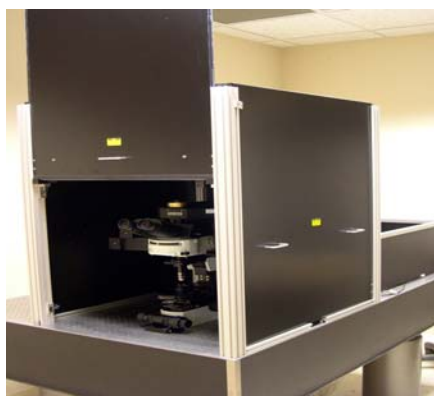


Figure 3.2: Ultima System with light box and beam cover

4 Hardware and Electronics

4.1 Overview

The Ultima Multiphoton Microscopy system contains many electronic and optical components. In this section, pertinent information regarding the electronics rack (shown in Figure 4.1) and key stage and imaging electronics will be described.

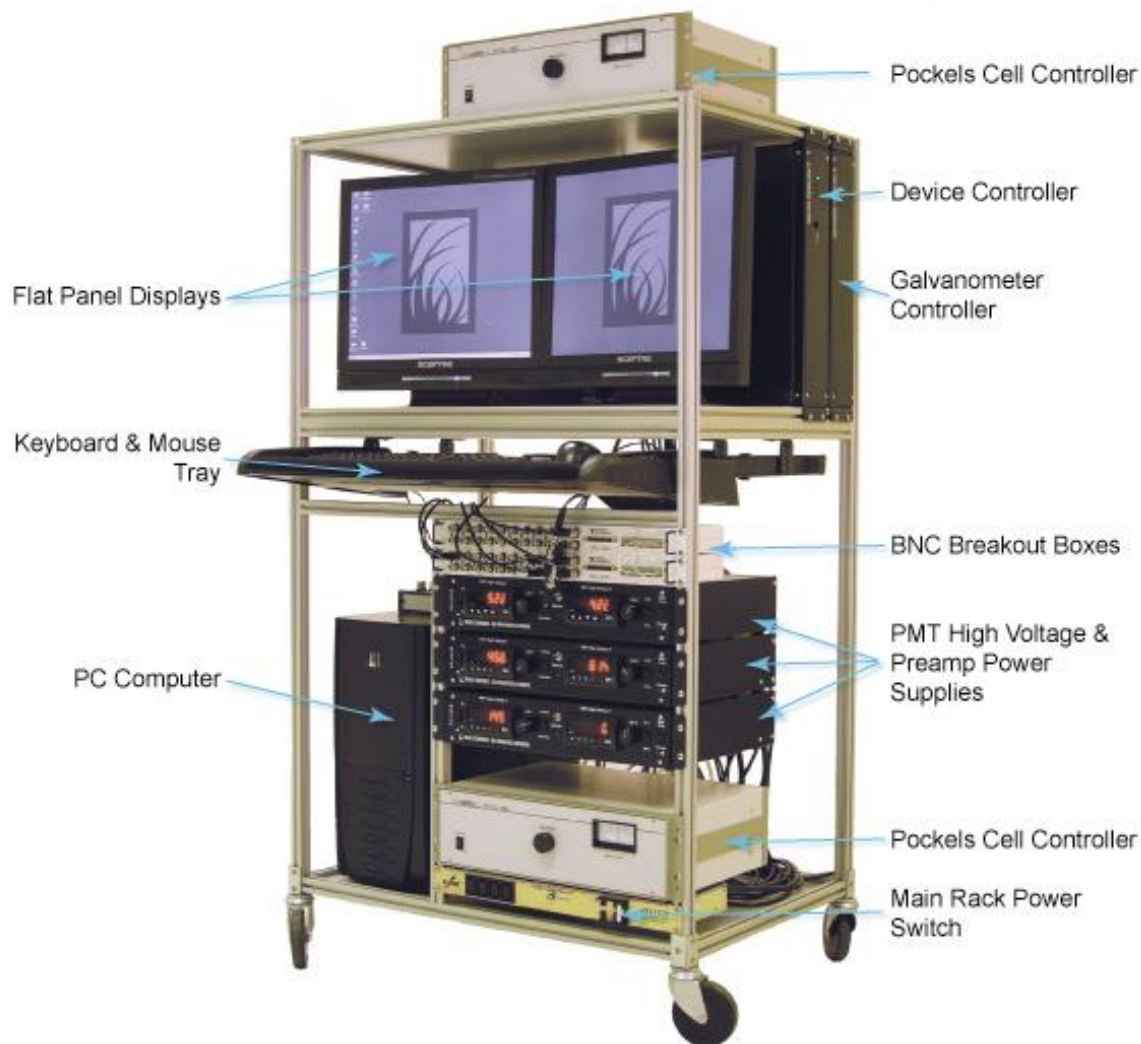


Figure 4.1: Ultima electronics rack

4.2 Rack and Other Electronic Components

The rack contains the majority of electronics necessary to operate the Ultima system.

4.2.1 PC

A PC is provided by Prairie Technologies which contains the three National Instruments PCI cards, software and drivers necessary to control the Ultima system.

4.2.2 PMT dual preamplifier & HV control unit

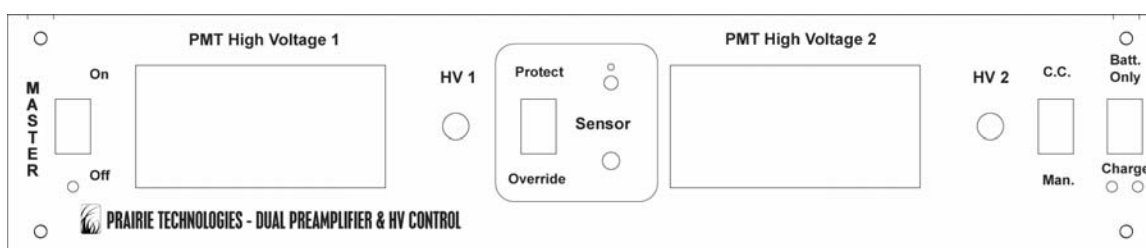


Figure 4.2: Dual Preamplifier & HV control unit

The PMT high voltage (HV) is controlled via the PMT control unit mounted in the instrument rack (shown in Figure 4.1). Each unit controls two PMTs. If your system has more than two PMTs you will have additional units in your instrument rack.

On the left side is the '**MASTER On/Off**' switch. This control is used to turn both PMTs on or off. The unit remains on unless the main power switch located on the backside is set to the '**Off**' position

The PMT high voltage may be controlled either via the computer or manually using the HV adjustment knob. Set the '**C.C./Man.**' switch to '**C.C.**' (computer controlled) or '**Man.**' (manual) as you prefer. Normally the system is configured to use the '**C.C.**' mode of operation.

If set to '**Man.**', the '**HV 1**' knob adjusts the high voltage supplied to PMT 1. The voltage is displayed in the red LED and the '**HV 2**' knob adjusts the high voltage supplied to PMT 2. In either computer controlled or manual mode the LEDs will display the HV of the PMTs.

The PMT HV control unit also has overload protection circuitry designed to prevent damage to the PMTs caused by exposure to an overly bright light source. The switch in the center of the unit labeled '**PROTECT/OVERRIDE**' is

used to either enable this feature (in the '**PROTECT**' mode) or disable it (the '**VERRIDE**' mode.) This mode only responds when the PMT becomes saturated. In order to reset the PMTs, reduce the cause of the saturation (the laser power may be too high, the PMT HV may be set too high, the room lights may be on, etc.) and then toggle the switch by moving it to '**VERRIDE**' and then back to '**PROTECT**'.

A second form of protection circuitry uses a photodiode sensor to monitor the ambient room lighting. When the '**SENSOR ON**' switch is in the up position the PMTs will be set to ~ 0 V when the room lights are turned on. If the room lights are then switched off the PMTs are turned back on. This mode of protection is only activated by external light sources.

Important Note: *The red LED display calibration buttons (arrow and 'Reset/Enter' buttons located directly below the LED displays) **should not be used**. These controls are used to calibrate the LED displays. They are factory set to display the correct high voltage values for each PMT but have no effect on the voltage itself. DO NOT CHANGE THESE SETTINGS, AS RE-CALIBRATION IS DIFFICULT AND TIME CONSUMING. If the display calibrations are inadvertently changed, they may be re-calibrated by referring to the supplied documentation on the Hawk brand meters.*

4.2.3 National Instruments BNC breakout boxes & boards

There are 3 National Instruments PCI boards in the system PC computer, the NI 6052E, NI 6713, and the NI 6110/6115.

There are also three external rack mounted National Instruments components, two BNC 2090 units and a single BNC 2110.

The NI 6052E and NI 6713 are connected together internally to share clock signals and other control signals. This results in highly synchronized operation. The 6052E board is used by *TriggerSync* to make electrical recordings. The 6713 board is used to generate output signals to various components as well as electrical stimuli for experiments. The 6110/6115 board is used to drive the imaging galvanometers and to collect PMT data.

4.2.3.1 NI 6052E board

The NI 6052E board collects all the input signals generated while running experiment. This board samples the enabled channels sequentially. If only one channel is enabled, the collection rate can be as high as 333K samples/sec. If additional channels are enabled for acquisition, (maximum number of acquisition channels is 8) the maximum sampling rate is divided down proportionally. Signals can be input to this board via the upper BNC2090 box in the electronics rack.

4.2.3.2 NI 6713 board

The NI 6713 board generates the output waveforms for the uncaging galvos, the Pockels cell, and the stimulus signals (maximum number of stimulus channels is 5) used in experiments. This board can generate a waveform at 1M samples/sec if only one channel is enabled. The maximum sampling rate drops as more channels are enabled. DAC5OUT and DAC7OUT are set aside for driving the uncaging galvos – the BNC2110 box has been modified for these two signals with the addition of voltage dividers that convert the +/-10V output range of these two signals to roughly +/-2.5V in order to increase the pointing resolution of the uncaging galvos in the region of use. The Pockels cell signal (currently DAC6OUT, although it can be changed) is similarly passed through a voltage divider to achieve better resolution in the 0-1.4V range of use except in this case the voltage divider is contained in a separate box external to the BNC2110 and the divide ratio can be adjusted if necessary by turning the screw of a potentiometer.

# of channels	1	2	3	4	5	6	7	8
Max input sample/sec for NI 6052	333K	166K	111K	83K	67K	56K	48K	42K
Max output samples/sec for 6713	1M	500K	333K	250K	200K	167K	143K	125K

Table 1: Maximum NI-6052 and NI-6713 sample rates

4.2.3.3 NI 6110/6115 Board – pixel data sampling methodology

The NI 6110/6115 board generates the output waveforms for the imaging galvos, and collects the PMT data used to generate images. There are four 12-bit input channels on the 6110/6115 board. Ultima systems that have the 6110 board sample at 2.5M samples/sec, while those with a 6115 board sample at 10M samples/sec. The pixel dwell time is a multiple of 0.4 μ s (6110) or 0.1 μ s (6115).

4.2.3.4 NI BNC-2090 & BNC-2110 boxes

There are two BNC-2090 boxes and one BNC-2110 box. The 2090 boxes are referred to as 2090/A and 2090/B for the upper and lower rack-mounted boxes, respectively. 2090/A is connected to the PCI-6052 card which is the acquisition card used by *TriggerSync*. 2090/B is connected to the PCI-6110 or PCI-6115 card which is the scan control/imaging card. The BNC-2110 box is connected to the PCI-6713 card which is the output card used by *TriggerSync*.

BNC-2090/A inputs ACH0-ACH7 are the eight *TriggerSync* input channels, DAC0OUT is unused. DAC1OUT is used in photoactivation. USER1 is the scanning shutter control signal and USER2 is the patch amplifier trigger input.

BNC-2090/B inputs ACH0-ACH3 are the four high speed ADC inputs of the PCI-6110 or PCI-6115 card (switches on each input must be set to the DIFF position.) DAC0OUT and DAC1OUT control the X/Y galvo in galvo systems or the AOD/Y galvo in AOD systems. USER1 is the Pockels cell blanking signal.

BNC-2110 outputs DAC5OUT and DAC7OUT control the X/Y galvos for uncaging. USER1 connects to the uncaging shutter. DAC0OUT-DAC4OUT and DAC6OUT are available through *TriggerSync* for other experiments.

The PDI0/TRIG 1 signal for all three breakout boxes should be connected together. This signal is the trigger signal that synchronized the start of an experiment/acquisition among the three NI cards. Normally these are also connected to the USER2 output of BNC-2090/B in which case the computer controls the start trigger.

Page 21 of 125

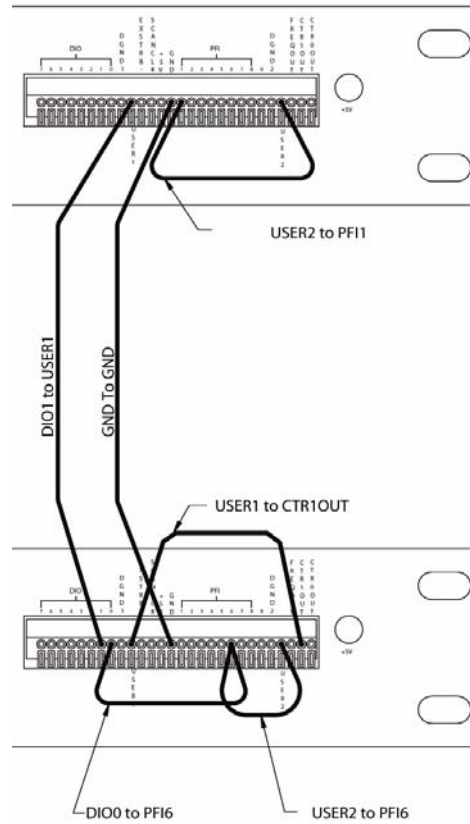


Figure 4.4: Close-up of NI BNC-2090 boxes

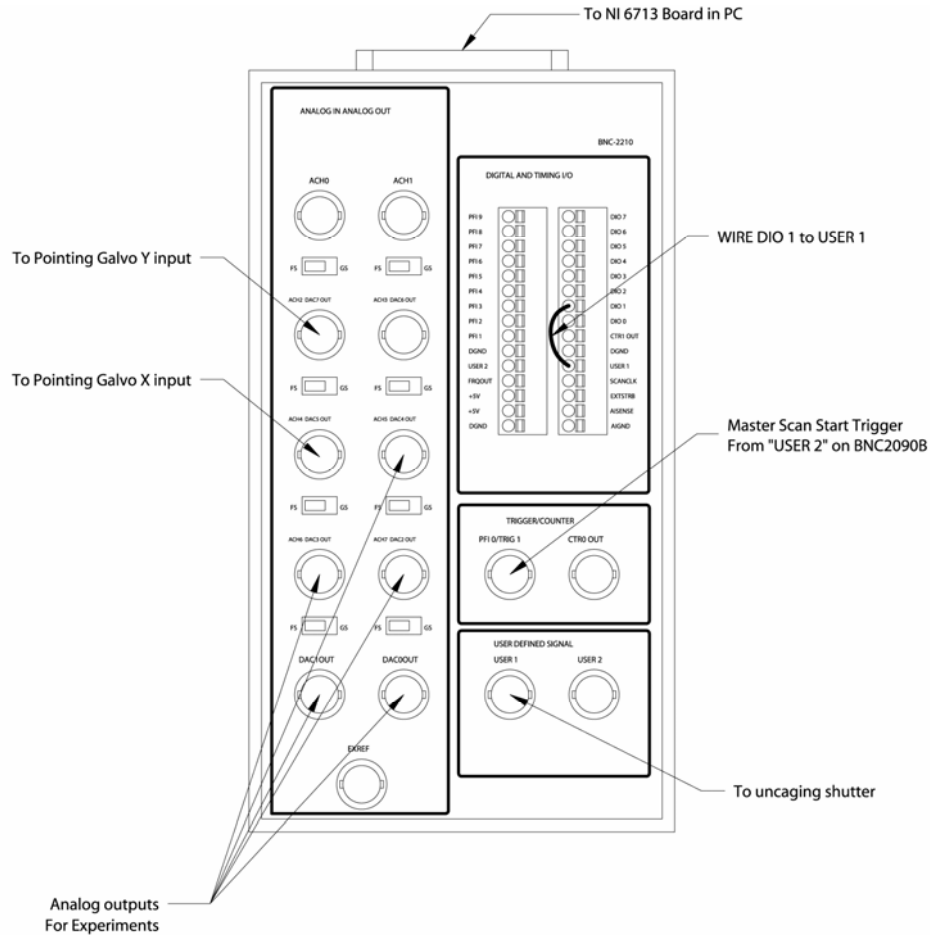


Figure 4.5: NI BNC-2110

4.2.4 Software communication through NI hardware

The software used for imaging, *PrairieView*, and the triggering software, *TriggerSync*, communicate by passing messages to each other using a TCP/IP protocol. In order for *PrairieView* to recognize that *TriggerSync* is running, *TriggerSync* must be started (or restarted) *after* *PrairieView* is in operation. Hardware synchronization of image collection and electrical recording is achieved by sharing a TTL trigger signal by the 6052, 6110, and 6713 boards (the PFI0/TRIG1 input on the upper BNC-2090, lower BNC-2090, and BNC-2110 box for the 6052, 6110, and 6713 boards, respectively). *PrairieView* and *TriggerSync* are triggered by a falling edge on PFI0/TRIG1.

4.2.5 Device Control Box and DCRI

NOTE: The following describes the use of the 'Mike's Machine' motorized X-Y stage and an Olympus BX-51 with automated Z-axis (fine focus) control. The step sizes for other configurations may vary from what is described below.

The motor system consists of the Prairie Device Control Box, the Device Control Remote Interface (DCRI) with three dials and eight toggle switches, the shielded motor cable, an RS-232 cable, and the motorized stage. All three motors have 200 full steps per revolution. The XY motors move the stage 1mm per revolution; the Z motor moves 0.1 mm per revolution. The dials on the DCRI produce 1000 counts per revolution. Two of the DCRI switches affect motor behavior; they are the '**Coarse/Fine**' and '**Normal/Alt**' switches. Coarse and fine settings vary depending on the installation; typical values might be 1/8th to 1/32nd stepping in coarse mode and 1/64th to 1/256th stepping in fine mode.

Assume a coarse setting of 1/16th stepping and a fine setting of 1/256th stepping in the following explanation. In coarse control mode, each count from the dials micro steps a motor 1/16 of a step (0.3125 μ m for the X-Y motors and 0.03125 μ m for the Z motor). In fine control mode, each count from the dials micro-steps a motor 1/256 of a step (~0.020 μ for X-Y motors and ~2 nm for the Z motor). When switching from fine to coarse control, any motors not already on a 1/16 step boundary first move to such a boundary using 1/256 step micro-steps before switching to coarse control. The normal/alt switch controls when the motors are enabled. In normal mode each motor individually disables itself after its most recent motion, and enables itself immediately before being ordered to move. When disabled, the motors do not resist being turned by hand and will not hold the stage stationary if other disturbances occur. In alt mode the motor is enabled continually whether or not it is moving.

X-Y fine mode:

$$(1000 \text{ micro-steps per knob turn}) * (1/256 \text{ step / micro-step}) * (1 \text{ motor rotation / } 200 \text{ steps}) * (1 \text{ mm / rotation}) =$$

$$\sim 19.5 \text{ } \mu\text{m/knob turn with a minimum step size of } \sim 19.5 \text{ nm}$$

For the Z-axis the figures are one tenth those above (only 0.1 mm / motor rotation):

Z coarse mode is 31.25 μ m per knob turn, 31.25 nm minimum step

Z fine mode is ~1.95 μ m per knob turn, ~1.95 nm minimum step

For all axes, the amount of motion for a single micro-step indicated above is an ideal estimate. The actual amount of motion will vary from micro-step to micro-step, due to internal factors within the motor, drive electronics inaccuracies, and friction within the mechanical hardware. Also, backlash will introduce positioning errors each time the direction of motion is changed. The most accurate positioning occurs at each whole step, as the motor naturally wants to sit at this position, and the electronics are not trying to balance the rotor between two phases (as they are required to do when micro-stepping).

Axis	Coarse Mode	Fine Mode
X	312.5 μm	19.5 μm
Y	312.5 μm	19.5 μm
Z	31.25 μm	1.95 μm

Table 2: Motor controller resolution

The Device Control Box contains power supplies, three micro-stepping motor drivers, and the logic for responding to commands from the DCRI or from the RS-232 input and for controlling the motors through the micro stepping motor drivers. Three LEDs on the front of the Device Control Box provide basic status information. The leftmost LED indicates power on, the center LED is on whenever the controller has moved any of its three motors within the previous half second. The rightmost LED indicates the status of the RS-232 send and receive lines. Traffic through the RS-232 port causes very brief, dim flashes on this LED. This LED will flash continuously 10 times per second if an RS-232 transmission error causes the input command buffer of the controller to overflow. In this state no further commands are recognized by the Device Control Box, the motors will halt, and the controller must be reset by either cycling power or pressing the reset button located on the front of the Device Control Box.

4.2.6 Prairie Preamplifier

The Prairie Preamplifier is a four-channel fixed gain, fixed bandwidth, variable offset summing preamp in an electrically isolated housing. The preamp requires supply voltages of $\pm 6.5\text{VDC}$ and a ground on a three conductor input connector. Preamp offset voltage is controlled through a USB connection. Each preamp channel has one BNC output and two BNC inputs. If both inputs for one channel are connected to photomultiplier tube outputs (PMTs) the signals are averaged. PMT gain is fixed at 10 $\mu\text{A/V}$ for a single input with a 3 dB bandwidth of 2.0 MHz using a 2-pole filter.

4.2.7 Galvos & Acousto-Optic Deflector (AOD)

The Prairie Galvanometer Control Box controls two Cambridge galvos using two Cambridge MicroMax 671 Drivers. Each galvo/driver combination comes as a set carefully tuned for best performance.



CAUTION

Caution: Switching cables so that the drivers control the wrong galvos can cause galvo damage.

In systems with a single galvo pair used for both scanning and uncaging the scanning inputs are connected to the 'X Input' and 'Y Input' BNC connectors and the uncaging inputs are connected to the 'X2 Input' and 'Y2 Input' BNC connectors. The 'Switch' BNC input is driver by the "TriggerSync" software to select between the scanning and uncaging control signals.

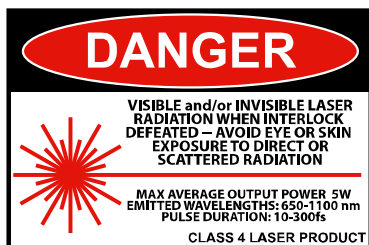
In systems with separate pairs of galvos for scanning and uncaging each pair uses the 'X Input' and 'Y Input' signals from its own Galvanometer Control Box.

Two amber-colored LEDs on the front of each box illuminate to indicate a shutdown state for the X and Y galvos, which occurs briefly on power up and afterwards only if the galvo experiences some failure such as being driven too hard so that it overheats. The 'X Feedback' and 'Y Feedback' are galvo position feedback signals which are used by Prairie during testing and configuration.

AOD systems will use the same Galvanometer Control Box. In AOD scanning mode the X galvo is held still and used for panning the image while the AOD scans in X.

4.3 Optics

A key part of imaging capabilities of the Ultima Multiphoton Microscopy system is the optics used.



WARNING

Never look into the laser beam path! The laser used in the Ultima system is a Class 4 infrared (IR) laser. It does not have a beam visible to the naked eye. The optics used in this system may also cause some back-scattered or reflected laser light. It is therefore critical to operate this system following all safety instructions and wearing appropriate laser-safe eyewear.

4.4 Laser Light Path

After exiting the laser, the beam of light travels to a Pockels cell modulator assembly (through a set of mirrors), which is an electro-optical modulator used for beam attenuation. At this point (via a pick-off window) approximately 4% of the laser beam is sent to a power meter so that laser power can be monitored. After passing through another set of mirrors and an interlocked electronic safety shutter, the beam passes into an enclosed beam-steering periscope assembly, which steers the aligned beam into the side car and then into the scan head. As it enters the scan head, the beam passes through an alignment iris and into the scan head (a self-contained unit not accessible to the user), where it passes through one or more matched pairs of galvanometers, optics, and optical components suitable for raster scanning a microscope image when coupled to an appropriate objective lens. The beam exits the objective. The beam is then scanned across the sample as a means of fluorescent excitation.

4.5 Ultima Scan Head

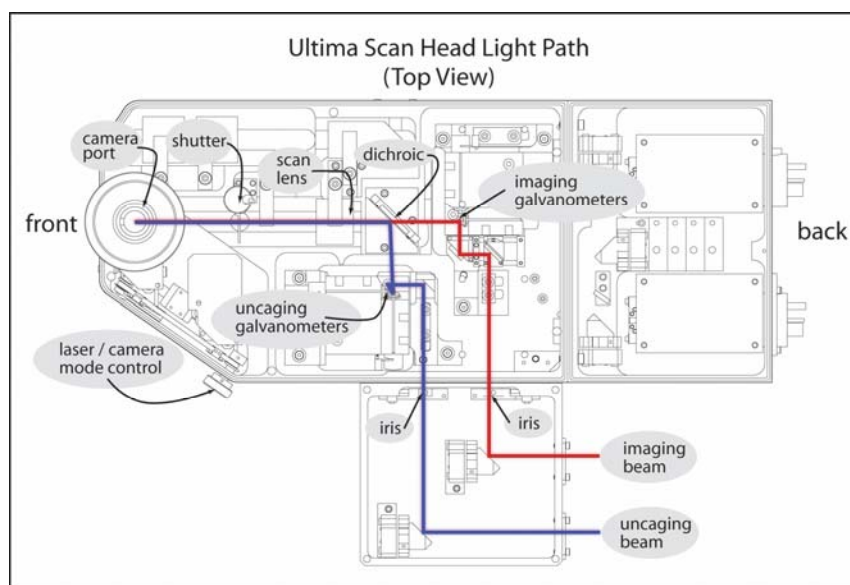


Figure 4.6: Ultima Scan Head light path

4.5.1 Dichroics

The Ultima has multiple user-changeable mirrors/dichroics, as shown in Figure 4.9:

Primary Dichroic: The **primary dichroic** is located in the Olympus epi-fluorescence illuminator in position 1. Based on the specific system configuration selected, this is a 660nm, 700 nm, or 720 nm LP dichroic. Additionally, there is a paired IR blocking filter located between the dichroic and the PMTs. The cube is custom-manufactured by Prairie.

PMT Dichroic: A **PMT dichroic** is based on a standard Olympus cube. For a dual detector, this dichroic includes a 575nm dcxr dichroic mirror and 607/45nm & 525/70nm barrier filters in front of PMTs No. 1 and No. 2 respectively. This combination was chosen to optimize dual labeling using Alexa 594 and Alexa 488. If removed entirely, all light is sent to PMT No. 1. For a quad detector, there are two PMT dichroics. The standard cubes that come with a quad detector are selected at the time the system is ordered and are designed to maximize the available wavelengths. To remove the PMT dichroics, pull down on the magnetic cover on the bottom of the housing.

A 100% mirror is supplied as standard equipment in the **Camera Port Mirror/Dichroic** position. The knob on the right hand side of the scan

head is used to move it in and out of position. When it is in position the Ultima operates as a laser scanner system. When it is retracted a normal CCD camera (customer supplied) may be used with standard trans-illuminated or epi-illuminated microscopy techniques. The mirror is in a mount manufactured by Prairie. It is also possible to mount a dichroic mirror in this position. Contact Prairie for more details.

The **Beam Combining Dichroic** is a 760nm dichroic used to combine the two 2-photon laser beams so that one is used to uncage and the other for imaging. The mirror is in a mount manufactured by Prairie.

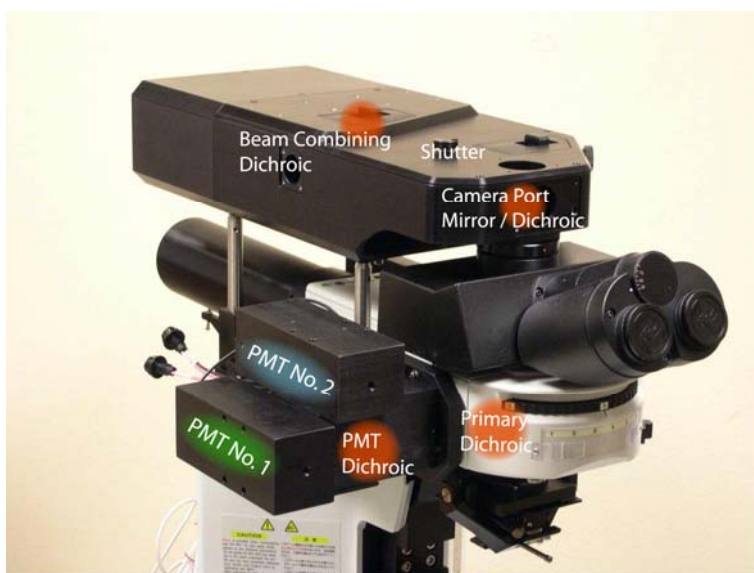


Figure 4.7: Ultima scan head dichroic locations

4.5.2 Microscope

The Ultima Multiphoton Microscopy System is built around a modified upright microscope. The manuals associated with this microscope have been provided to the user. Please refer to these manuals for specific information about the microscope components

4.5.3 Detectors



CAUTION

PMTs are extremely sensitive to light. Care should be taken to eliminate all sources of stray light from reaching the PMTs, as this will dramatically reduce the sensitivity of the system. Prairie Technologies provides a light-tight and laser-

safe enclosure around the instrument to ensure optimal performance as well as to provide a safe working environment for the users of this instrument.

Note: Only four detectors may be used simultaneously.

4.5.3.1 Top-Mounted detectors

The Ultima system comes with either dual or quad non-descanned top-mounted detectors. They may be either the standard side-on multi-alkali PMTs or (optional) GaAsP detectors. The emission pathway for both options is similar and is depicted in Figures 4.10 and 4.11. In both cases, the fluorescence from the sample is reflected off the primary dichroic in the turret, through the IR-blocking filter and through the various dichroics and barrier filters to the PMTs.

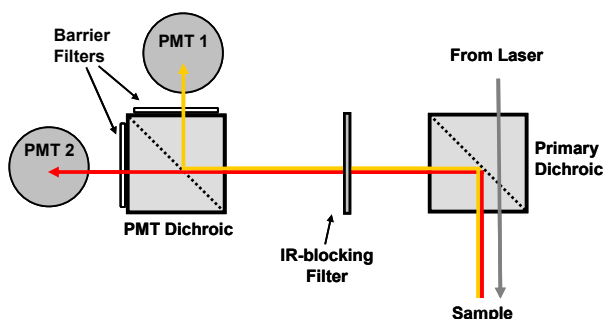


Figure 4.8: Emission Light Path for dual-channel detectors

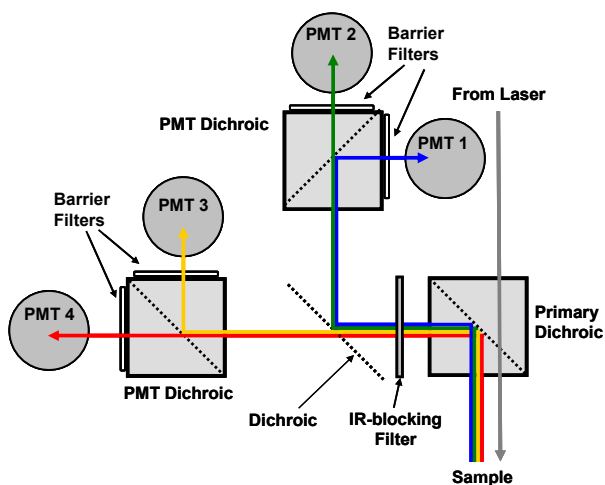


Figure 4.9: Emission Light Path for quad-channel detectors

4.5.3.2 Sub-Stage detectors

Some systems come with the optional pair of sub-stage multi-alkali PMTs. These detectors are mounted below the stage and to the left of the microscope.

4.5.3.3 Dodt Gradient Contrast detector

Some systems come with the optional transmitted light Dodt gradient contrast detector. This detector is mounted at the back of the microscope and is used for obtaining 'DIC-like' images, which are useful for imaging unstained cells (for example, in electrophysiological experiments involving patch-clamping).

5 Ultima Startup Procedure

- Following instructions from laser manufacturer, put laser in “ready” state.
- Turn on the electronics rack.
- Check and/or install filters and dichroics into upper PMT detection path. (BG39, Olympus cube and filters.)
- Turn on the PC (**Important: Disable anti-virus software**).
- Close Sub-stage detector shutter. (Sub-stage detectors are optional and may not be present in your system.)
- Start *PrairieView*.
- Start *TriggerSync* if you plan to conduct electrical recording experiments.
- Verify the communication link between *PrairieView* and *TriggerSync*. A message will appear at the bottom of the *PrairieView* window stating that the link has been established.
- Open laser shutter in laser software (provided by laser manufacturer).
- Push Olympus trinoc plunger in, to the ‘**Bi**’ position.
- Position Olympus epi-dichroic wheel to some open position. (Transmitted light will turn on automatically.)
- Put sample on stage and focus.
- Turn off or COMPLETELY shutter the epi-fluorescence mercury lamp house if present on your system. The “Shutter” mechanism provided on the front of the Olympus epi-fluorescence illuminator is NOT an adequate shutter as it leaks light from the lamp house that will flood the PMTs.
- Turn Epi dichroic wheel to ‘**Position 1**’ (This automatically turns off the Olympus transmitted light source, and directs signal to the upper PMTs.)
- Pull Olympus trinoc plunger out, the ‘**LSM**’ position.
- To use sub stage detectors (if present):
 - Turn on sub-stage High Voltage Power Supply.
 - Plug appropriate sub stage detector PMT cable into PMT junction box.
 - Install the same filters and dichroics into sub stage detectors as are in the upper detectors.
 - Slide sub stage dichroic in position.
 - Open sub stage condenser iris.
 - Position sub stage condenser.
 - Open sub stage shutter.
- Turn off the room lights.
- Note the laser power reading on the power meter, adjust if necessary using the Pockels cell control in *PrairieView*.
- Use *PrairieView* to open one or more imaging windows.
- Press ‘**Live Scan**’ button. This starts the scanning, and opens the laser shutter.
- Turn up the PMT high voltage until you see your image.

6 Alignment and Calibration Procedures

6.1 Beam Alignment

Important: *The optical path for the laser beam has been carefully and precisely aligned. Re-aligning the laser beam may be necessary only under the following circumstances and should be performed only by a factory-trained and authorized technician:*

- 1) The direction of the laser beam out of the laser changes.
- 2) A mirror on the tabletop is adjusted.
- 3) A Pockels cell is added to or removed from the light path.
- 4) Something new is added to the light path that would cause the direction of the beam entering the Ultima to change.



WARNING

Never look into the laser beam path! *The laser used in the Ultima system is a Class 4 infrared (IR) laser. It does not have a beam visible to the naked eye. The optics used in this system may also cause some back-scattered or reflected laser light. It is therefore critical to operate this system following all safety instructions and wearing appropriate laser-safe eyewear.*

6.1.1 Imaging Beam Alignment in Scan Head

Important: *Re-aligning the imaging laser will introduce an offset into all of the spot calibration files.*

- Close *PrairieView*.
- Close *TriggerSync*.
- Remove sample.
- Raise objective lens all the way up.
- Install objective target in turret.
- Open measurement and automation toolbox on windows desktop, under:
 - Devices and Interfaces.
 - Traditional NI-DAQ devices.
 - Select PCI-6110.
 - Select Test Panels.
 - Use analog output and DC voltage.
 - Set channels 0 and 1 to 0.00 Volts. Be sure to press update channel after selecting each channel.

- Close shutter for uncaging beam.
- Open shutter for imaging beam.
- Verify that the image of the X-axis galvo mirror is centered on objective target ring.
- Use adjustments at top of tower to center beam at first pinhole.
- Use adjustments in sidecar to center spot on objective lens target.
- Stop iris down only to the size of the ring in the objective target window.
- Leave iris in this position to minimize back reflections into upper PMTs.
- Close measurement and automation.
- Return Uniblitz shutter switches to their normal positions.
- Restart system software.

6.1.2 Uncaging Beam Alignment in Scan Head

Important: *Re-aligning the uncaging laser will introduce an offset into all of the spot calibration files.*

- Close *PrairieView*.
- Close *TriggerSync*.
- Remove sample.
- Raise objective lens all the way up.
- Install objective target in turret.
- Open measurement and automation toolbox on windows desktop.
- Under
 - Devices and Interfaces.
 - Traditional NI-DAQ devices.
 - Select PCI-6713.
 - Select Test Panels.
 - Use analog output and DC voltage.
 - Set channels 5 and 7 to 0.00 Volts.
 - Be sure to press update channel after selecting each channel.
- Close shutter for imaging beam.
- Open shutter for uncaging beam.
- Verify that the image of the X-axis galvo mirror is centered on objective target ring.
- Use adjustments at top of tower to center beam at first pinhole.
- Use adjustments in sidecar to center spot on objective lens target.
- Stop iris down only to the size of the ring in the objective target window.
Leave iris in this position to minimize back reflections into upper PMTs.
- Close measurement and automation.
- Return Uniblitz shutter switches to their normal positions.
- Restart system software.

6.2 Spot Detector Alignment

Important: This is normally a one-time adjustment. The only time this will need to be performed is if either of the galvos is replaced, a mirror in the scan head is moved, or the beam combining dichroic in the scan head is replaced

- Put reflective chrome slide on stage.
- Start PrairieView.
- Enable channel 1 in PrairieView.
- Start TriggerSync.
- Verify the communication link between PrairieView and TriggerSync. A message will appear at the bottom of the PrairieView window stating that the link has been established.
- Close uncaging shutter in the TriggerSync Mark Points window.
- Image the slide with imaging beam.
- Activate mark points screen. Make sure image in mark points screen is updating.
- Manually toggle shutter on uncaging beam to burn small spot on chrome slide.
- Put red TriggerSync cursor over mark on chrome slide. (Do not use “nudge” button.)
- Turn off channel 1 and turn on channel 3.
- Close shutter on top of Ultima.
- Open uncaging shutter in TriggerSync.
- Activate focus.
- Move uncaging spot to be coincident to red TriggerSync cursor.
- Close uncaging shutter in TriggerSync.
- Open shutter on top of Ultima.
- Turn off channel 3 and turn on channel 1.

6.3 Calibrate Point Image

Important: Each objective lens will require its own unique calibration file.

- Close imaging beam shutter.
- Start PrairieView.
- Start TriggerSync.
- Verify the communication link between *PrairieView* and *TriggerSync*. A message will appear at the bottom of the *PrairieView* window stating that the link has been established.
- Turn off channels 1 & 2.
- Turn on channel 3.
- Set to 4ms/line.
- Open Uncaging laser shutter in *TriggerSync*.
- Perform 'Point Calibration' as described in Section [8.4](#).

6.4 Resetting the PMT High Voltage

Important: The PMT high voltage will automatically turn off if an overload condition is detected.

The system is designed with two levels of PMT protection. First is the photodiode that is located in the PMT high voltage power supply. This senses when the room lights are on and automatically turns the PMT high voltage to 0 V. The second protection is built in to the signal detection electronics. When an excessively bright signal is detected the PMT high voltage is again set to 0 V.

To reset the PMT high voltage power supply:

- Correct the condition that caused the overload condition to occur.
- Turn the '**Protect/Override**' toggle switch on the front of the PMT high voltage power supply momentarily to '**Override**'
- Turn the '**Protect/Override**' toggle switch on the front of the PMT high voltage power supply back to '**Protect**'.

This will cause the PMT high voltage power supply to return to its pre-overload values.

Important: When an image is not visible (or suddenly disappears), it may be due to this PMT HV safety feature.

7 *PrairieView* Software

PrairieView is the software that controls all of the scanning and image collection functions of the Ultima. Image size, scan rate, pan, zoom, scan rotation, PMT settings, laser wavelength and power level, file saving and naming are all set and easily controlled using *PrairieView*. This acquisition software also offers a number of features to meet most imaging needs including scan rotation, line scan, brightness over time (BOT), region of interest (ROI), Z-series, photoactivation, marked points, and T-series.

7.1 *PrairieView* - Main Window

Once the Ultima and the laser have been powered up, the operator is ready to start *PrairieView* by clicking the icon located on the desktop. The main program screen and one Image Window will appear, as shown in Figure 7.1.

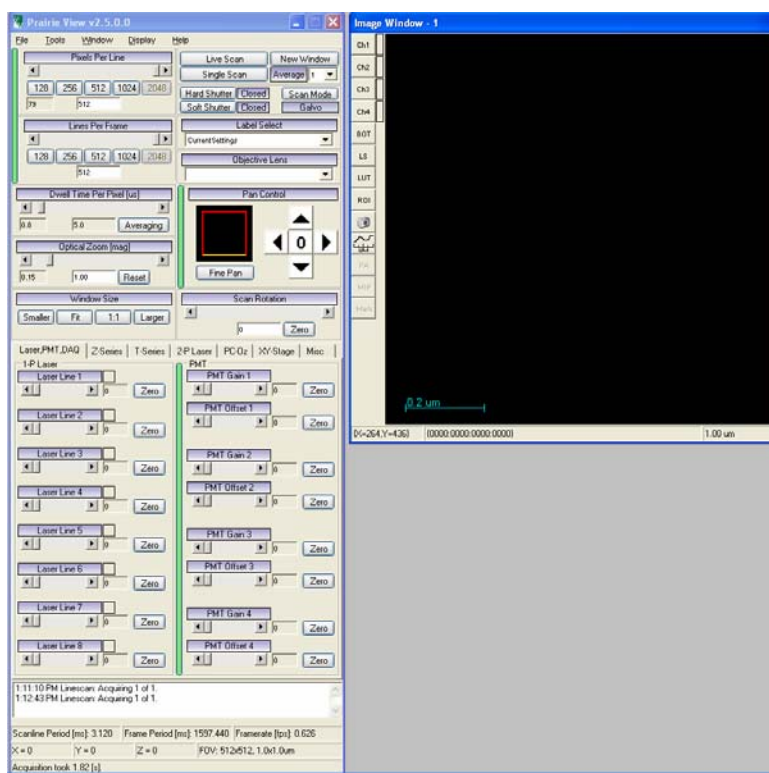


Figure 7.1: *PrairieView* Main Window.

7.2 Main Window Controls

Most of the controls of *PrairieView* are accessed through the Main Control Window.

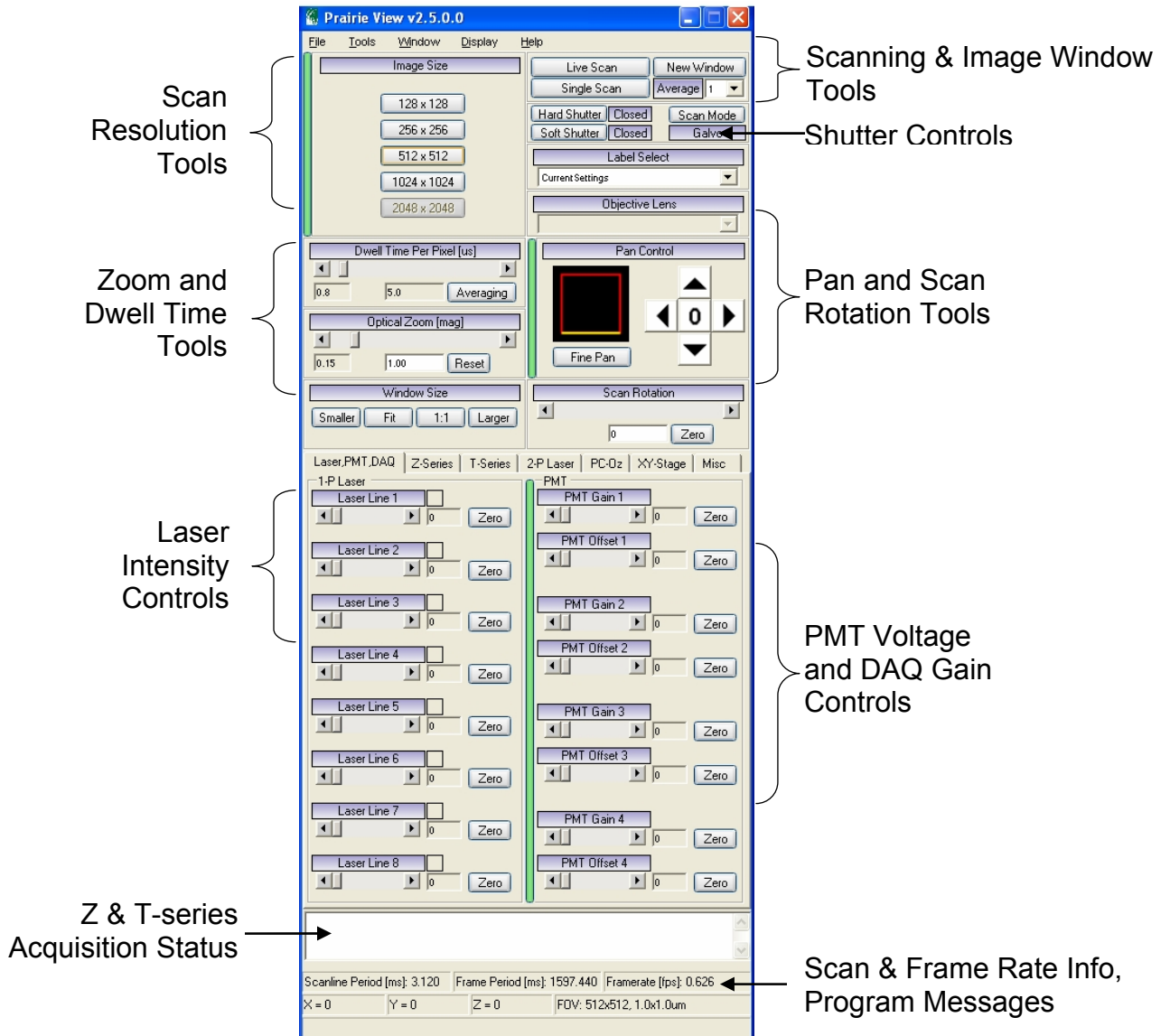


Figure 7.2: *PrairieView* Main Screen.

7.2.1 Scan Resolution Tools

The green vertical bar on the left of this section allows the user to switch between the standard square image scan choices and independent selection of the pixels per line and lines per frame

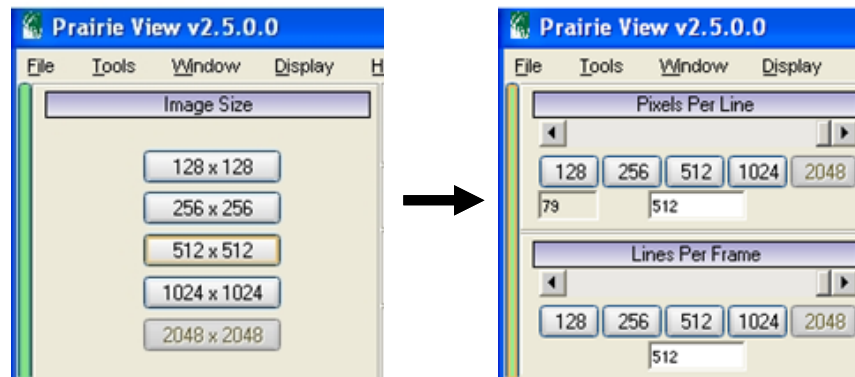


Figure 7.3: Scan resolution tools.

7.2.1.1 Image Size

Image size is a definition of how the collected data is acquired (and displayed). In the case of 512 x 512 for example, data is acquired at 512 x 512 bins, with each bin sampled according to dwell time.

7.2.1.2 Pixels Per Line & Lines Per Frame

Pressing the green bar to the left of the Image Size window section activates this mode.

The 'Pixels Per Line' function allows the user to define the number of pixels to dwell upon in each horizontal line (X – direction) of the image. Preset values are offered as a convenience. Values may also be selected by means of the slider tool or by typing directly into the highlighted field. The minimum value allowed is displayed to the left of the highlighted field.

The 'Lines Per Frame' function allows the operator to define the number of lines (in the Y-direction) in each image. Preset values are offered as a convenience. Values may also be selected by means of the slider tool or by typing directly into the highlighted field.

Note: Be aware that it is possible to choose configurations in which the pixels are not square. This may effect how the images are displayed by third party software.

7.2.2 Zoom and Dwell time Tools

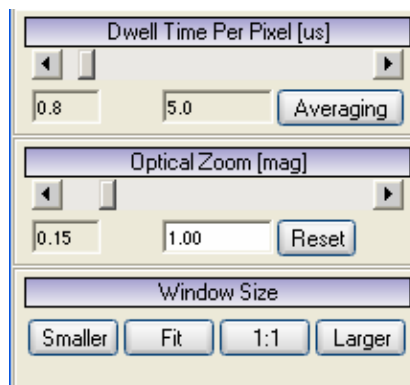


Figure 7.4: Zoom and dwell time tools.

7.2.2.1 Dwell Time Per Pixel

Controls the dwell time that the laser beam is at each pixel location in the image, with values in microseconds (μ s). The minimum value allowed is displayed below and to the left of the slider. Increasing pixels per line, zooming or rotating may decrease the minimum dwell time possible.

7.2.2.2 Optical Zoom

Controls the size of the area of the specimen that is being scanned. An optical zoom = 2 will cause the microscope to scan an area that is $\frac{1}{2}$ the width and $\frac{1}{2}$ the height of a scan at a zoom = 1. Optical Zoom does not affect the number of pixels in the image. It is possible to increase the true optical resolution of the system by using this function. The 'Reset' button causes the zoom to return to the default value = 1.) The minimum value allowed is displayed below and to the left of the slider.

Note: It is not possible to set the optical zoom to less than 1.

7.2.2.3 Window Size

There are four options under 'Window Size'.

- 'Fit' is a default to 512 x 512.
- '1:1' means that one display pixel is equal to one acquisition pixel.
- 'Smaller' decreases the image size by approximately 10%.
- 'Larger' increases image size by approximately 10%.

7.2.3 Scanning & Image Window Tools

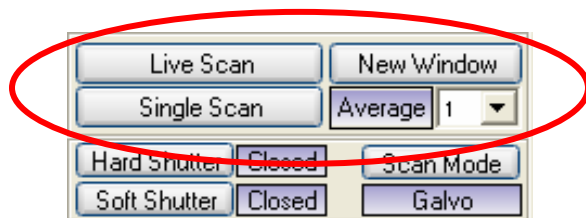


Figure 7.5: Scanning tool.

7.2.3.1 Live Scan/Stop Scan

To start the Ultima system scanning click on '**Live Scan**'. While a live scan is being performed this button changes to read '**Stop Scan**'. Clicking a second time causes the scan to stop and the laser beam to be shuttered so that it no longer scans across the specimen.

7.2.3.2 Single Scan

When '**Single Scan**' is clicked, the laser is scanned once across the specimen with the result displayed in the image window(s). The laser is shuttered following collection of the image.

7.2.3.3 New Window

Clicking on '**New Window**' will cause another image window to appear on the desktop. It is possible to have multiple image windows open at one time. The operator can, for example, assign a different PMT to separate image windows if desired.

7.2.3.4 Average/Sum

During data acquisition, the underlying sampling rate is 1 sample per 0.4 μs for a galvo system. This means that for a 4 μs dwell time, the system is either averaging or summing $4 \mu\text{s} / 0.4 \mu\text{s} = 10$ samples per bin.

7.2.4 Shutter and Imaging Mode

7.2.4.1 Hard Shutter Closed/Open

Although software controlled for routine imaging, it is possible to manually open and close the hard shutter to allow access to the beam by *TriggerSync* or other interfaced applications.

7.2.4.2 Soft Shutter Closed/Open

Although software controlled for routine imaging, it is possible to manually open and close the soft shutter. This is most useful when the mechanical movement of the hard shutter causes disruptive vibration during electrophysiological experiments.

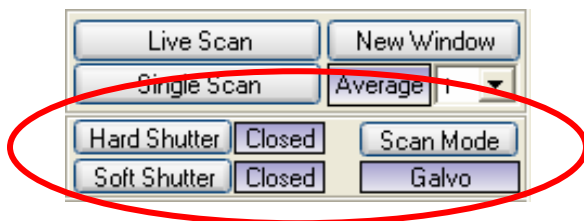


Figure 7.6: Shutter and imaging mode.

7.2.4.3 Scan Mode: Galvo or AOD

This button allows the user to select between the standard galvo imaging mode and the optional AOD imaging mode.

7.2.4.4 Label and Objective Lens Selection

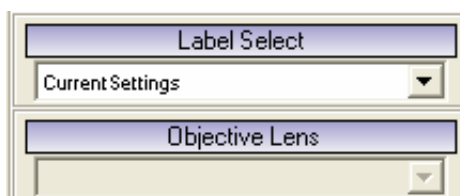


Figure 7.7: Label and objective lens selection.

7.2.4.5 Label Selection

Label selection allows the user to select previously defined and saved experimental protocols. These protocols include saved settings for active channels, PMT selection and DAQ gain, laser power settings. Setting up labels is covered in Section [7.2.11](#).

7.2.4.6 Objective Lens

Objective Lens allows the user to select a previously calibrated objective setting for an objective used on the system. The procedure for calibrating objective lenses can be found in Section [7.3.2.1](#).

Note: Proper objection selection is important as it is used in determining pixel size. Failure to select or use a properly calibrated objective will result in invalid measurements.

7.2.5 Pan and Scan Rotation Tools

7.2.5.1 Pan Control and XY Stage Control

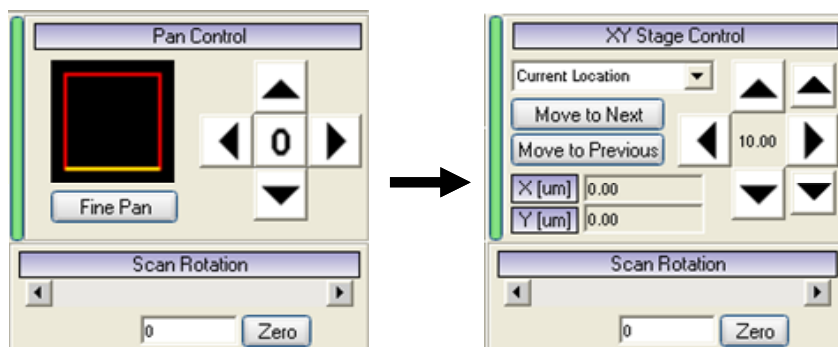


Figure 7.8. Pan Control & XY Stage Control.

Below the Pan Control banner are two sets of icons. On the left is an indicator that shows the relative position and orientation of the scanned area. A red and yellow box indicates the scanned area; the yellow side is by definition the “bottom” side. This box will change its size as a result of the Optical Zoom function described above. Similar changes will be displayed when the image is panned, or shifted laterally, and when the scan is rotated.

The ‘**Coarse/Medium/Fine**’ button controls the feel or resolution of the pan controls.

The image may be panned left, right, up, or down. To do so, click on the arrow indicating the direction to pan the image. Clicking the ‘0’ in the center causes the pan to reset to the middle of the field of view.

7.2.5.2 Scan Rotation

Scan rotation is controlled by means of the slider, or values (in degrees) may be typed directly into the highlighted field. Rotation is possible from -180° to $+180^{\circ}$.

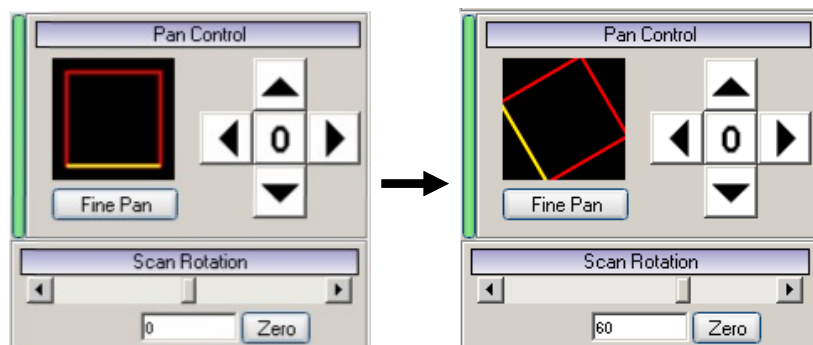


Figure 7.9: Scan Rotation.

7.2.5.3 XY Stage Control

The XY Stage Control allows the user to move the stage in the x, y, and z directions. It also allows the user to recall saved locations. The step size is shown in the center of the arrows and has a default of 10 μm . This can be adjusted in the XY-Stage tab (see Section [7.2.10](#))

7.2.6 Laser, PMT, DAQ

This tab, located at the left center of the Main Control Window, enables adjustments that control the laser power to reach the specimen and the gain setting of the DAQ data acquisition board in the computer.

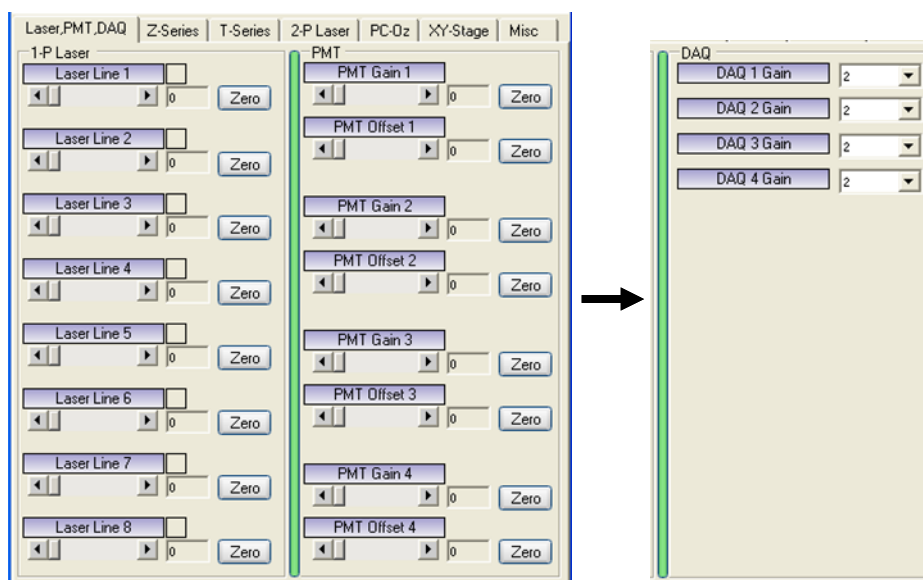


Figure 7.10: Laser, PMT, DAQ Controls.

7.2.6.1 Laser

The 'Laser' slider is used to adjust the laser intensity that reaches the specimen. In most Ultima systems the imaging beam intensity is controlled via a Pockels cell that is tied to this control. If your system has two lasers then a second slider will be activated to control a second Pockels cell for the uncaging laser intensity.

7.2.6.2 PMT Gain (voltage)

The sliders are used to set the PMT high voltage for each detector in the system. The text field to the right displays the voltage that corresponds to the red LED displays on the Dual Preamplifier & HV Control Unit (Section [4.1](#)) in the instrument rack. The 'Zero' button sets the voltage to ~ 0 V.

7.2.6.3 DAQ Gain

The NI DAQ 6110 board in the computer has a gain setting that is applied to the PMT pre-amp output voltage which is the “signal” from your detectors. The drop down arrow is used to select the desired gain value from the list of choices.

7.2.7 2P Laser

The 2P Laser tab provides additional options for laser control of the Ultima’s laser(s) including laser selection and wavelength adjustment.

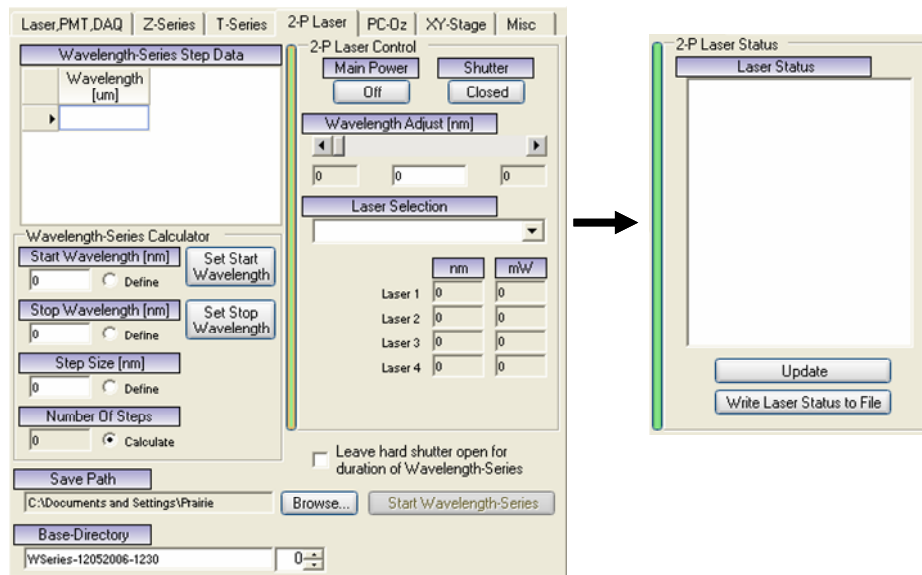


Figure 7.11: 2P Laser Control.

7.2.8 Z-series

Under the ‘Z-series’ tab are the controls that are used to collect stacks of images called Z-series or z-stacks, and to directly control the z-motor when searching for a desired focal plane in the specimen. This tab allows the user to define start and stop positions, step size or number of steps and necessary PMT adjustments.

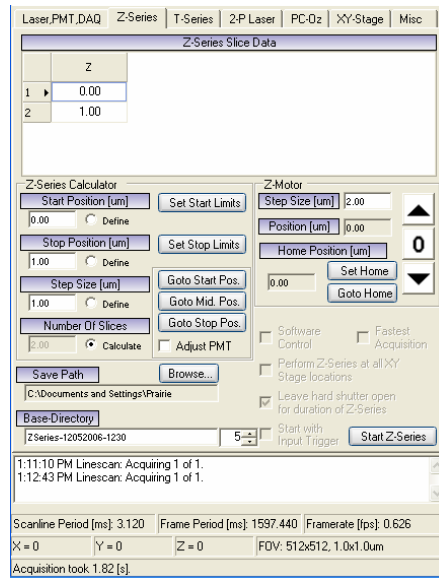


Figure 7.12: Z-series set-up.

7.2.8.1 Z-Motor Control

The Z-Motor control allows the user to scan through the z-axis of an image in user-define step sizes.

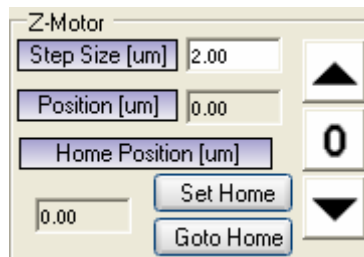




Figure 7.13: Z-Motor control.

Use the  and  arrows to move the z-motor by the number of μm indicated in the 'Step Size' field. To change the step size, click in the field, replace the number with the desired step size, and press <Enter>. The step size value in this field is NOT used during acquisition of a Z-series.

7.2.8.2 Z-series Calculator

In order to collect a Z-series stack the operator must define several parameters that define the range, resolution, and signal levels. Four values are needed to define the range and resolution of a Z-series: Start Position, Stop Position, Step Size, and Number of Slices or image planes. By entering values for any three of these values, the fourth value will be calculated automatically. The

'Define/Calculate' radio buttons indicate the three values that the operator must 'define' as well as the one that is calculated by the system.

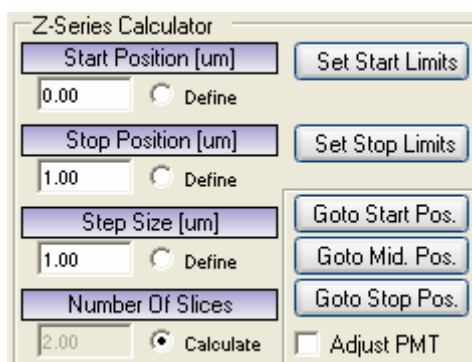








Figure 7.14: Z-series calculator.

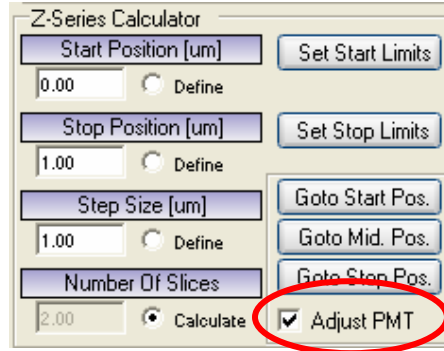
To collect a Z-series in which the system calculates the number of slices (images) to collect:

- Use the  and  arrows to move the z-motor to the correct focal plane for the starting position of the Z-series.
- Press '**Set Start Limits**'.
- Use the  and  arrows to move the z-motor to the desired focal plane for the **ending** position of the Z-series.
- Press '**Set Stop Limits**'.
- Enter the desired '**Step Size**' if different from the distance displayed. Click on the field, type the new distance, press <Enter>.
- Confirm that the 'Save Path' and 'Base-Name' are correct. This defines where the images will be saved.
- Press '**Start Z-series**'.
- Acquisition of the Z-series can be monitored in the window below the 'Start Z-series' button.

7.2.8.3 Collecting a Z-series over a large depth

When collecting a Z-series over a large depth it is sometimes desirable to have different laser intensity and or PMT voltage settings at each level. For example more laser power may be needed to penetrate and provide sufficient signal levels at great depths in the specimen. *PrairieView* provides such a mechanism. In order to have a nonlinear PMT change applied to the laser intensity and/or the PMT HV, simply follow the steps outlined below:

- Use the  and  arrows to move the z-motor to find the desired focal plane for the start and stop position of the Z-series.
- Turn on 'Adjust PMT' in the Z-series Calculator window:



- Click on **'Go to Start Pos.'**
- In the Laser, PMT, DAQ tab, set desired laser and PMT settings.
- Click on **'Set Start Limits'**.
- Click on **'Go to Stop Pos.'**
- In the Laser, PMT, DAQ tab, set desired laser and PMT settings.
- Click on **'Set Stop Limits'**.
- Each line in the Z-Slice Data Window will reflect the laser and PMT settings as calculated by *PrairieView*.

7.2.9 T-series

The ability to generate a series of images and data using different labels, region-of-interest (ROI) settings, XY stage coordinates, Z-series, photoactivated regions, and triggers are possible using the T-series tab. This allows the user to optimize a complex set of experimental parameters to the acquisition of key data.

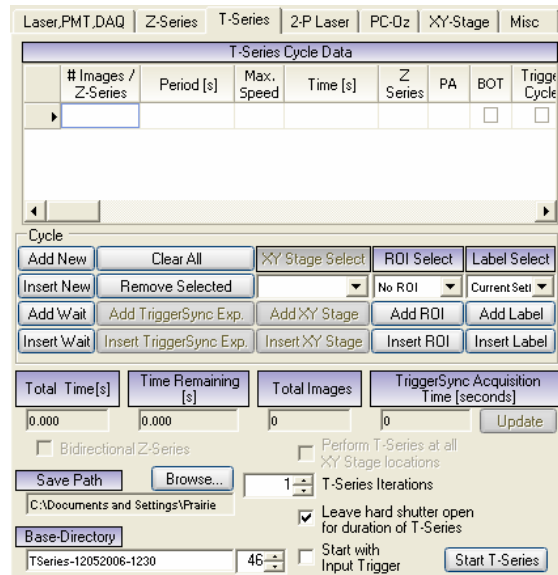


Figure 7.15: T-series tab.

7.2.9.1 T-series definitions

Images/Z-series: How many images or Z-series are to be acquired at this position.

Period: The length of time *it takes to go from start to start in* a collection of images or stacks.

Max Speed: A mode which allows images to be acquired continuously (there is no live update while this acquisition is occurring). Max Speed is available for Z-series only with piezos.

Time: Minimum amount of time to acquire the image(s); calculated by PrairieView.

Z-series: Acquire a Z-series instead of a single image (See Section [7.2.8](#)).

PA: Use predefined photoactivation (PA) masks corresponding to a z-slice (See Section [7.4.7](#)).

BOT: Collect brightness-over-time (BOT) data based on predefined regions(See Section [7.4.2](#)).

Trigger Cycle: Wait for input trigger before processing line.

Trigger Each Image/Z-series: Wait for trigger before each image or Z-series.

Cycle Completion: Function not used at this time.

Add New: Place a new basic cycle at end of series that can be modified for Z-series/PA/BOT/trigger.

Insert New: Insert a new basic cycle in series at position pervious to marked line that can be modified for Z-series/PA/BOT/trigger.

Clear All: Function not used at this time.

Remove Selected: Removes selected lines from T-series.

Add/Insert *TriggerSync* Exp: When a *TriggerSync* is running these buttons become enabled. When included in a T-series they will cause *TriggerSync* to perform an 'Acquire' operation as defined in the current

XY Stage/ROI/Label Select: Apply to all subsequent relevant lines in the T-series until changed (See Section [7.210](#) for XY Stage, Section [7.4.5](#) for ROIs, and Section [7.2.11](#) for Labels).

Save Path/Base Directory: A default path and base directory is set up for saving data. This is C:\Documents and Settings\Prairie with a Base Directory name that includes the type of data acquired, the data of acquisition, and a file number that is sequentially incremented. If a different file name or location is preferred, click '**Browse**' next to 'Save Path' to change this information.

7.2.9.2 Collect a Basic T-series

A **Basic T-series** is one in which an image or Z-series is collected at a desired interval.

- Press the '**Add New**' button. A row with default settings will be created.
- To select the number of images or Z-series to acquire, click on the '**# Images/Stacks**' field, type the desired number of images to collect, and press <Enter>. The default value is 1, so no changes are necessary if only one image or Z-series is desired.

-
-
- Click on the **'Period'** field, type in the time required for this line of acquisition to take. Press <Enter>
 - Click the **'Max Speed'** check box, if this is desired.
 - Click the **'Z-series'** check box. This will cause a Z-series to be collected as currently defined in the Z-series tab (Section [7.2.8](#)).
 - Verify the Save Path.
 - Press **'Start T-series'**.

Note: The “active” T-series line is the one marked by an arrow in the leftmost column of the T-series cycle data list.

7.2.9.3 Collect a Complex T-series

A Complex T-series is one in which numerous steps defining different operations are strung together into a single experiment. It is possible to collect a Z-series at a set interval or to collect individual images and Z-series alternately.

For example, a user wants to create a complex T-series in which there are three steps; 1) collection of 10 single images with 30 seconds between each image, 2) a waiting period of 4 minutes, and 3) the collection of a Z-series.

To create a Complex T-series such as this:

- Press the **'Add New'** button. A row with default settings will be created.
- To select the number of images or acquire, click on the **'# Images/Stacks'** field, type the desired number of images to collect, and press <Enter>.
- Click on the **'Period'** field, type in the time required for this line of data acquisition to take. Press <Enter>
- Click the **'Max Speed'** check box, if this is desired.
- Press **'Add Wait'**. A second line appears.
- Click on the **'Period'** field, type in the total time for the system to wait between the preceding and following acquisitions. Press <Enter>
- Press **'Add New'**. A third line appears.
- Click the **'Z-series'** check box. This will cause a single Z-series to be collected as currently defined in the Z-series dialog.
- Verify the Save Path.
- Press **'Start T-series'**.

Note: The “active” T-series line is the one marked by an arrow in the leftmost column of the T-series cycle data list.

7.2.10 XY Stage

The XY Stage tab allows the user to select and save specific x, y, and z coordinates. This makes it possible to return to locations of interest in the sample and is especially useful in setting up complex experiments.

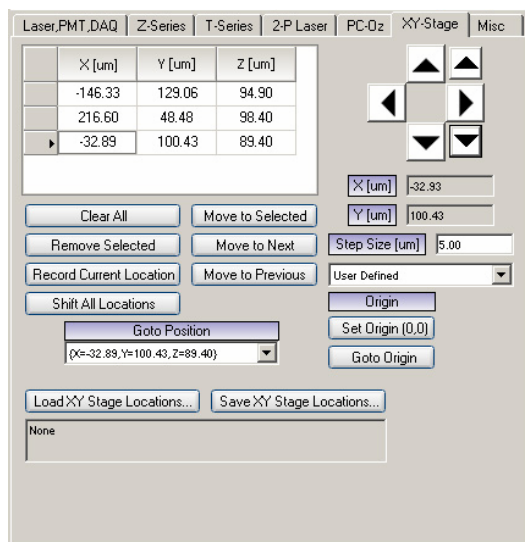


Figure 7.16: XY-Stage tab.

7.2.10.1 XY-Stage definitions

Clear All: Function not used at this time.

Remove Selected: Removes selected lines from T-series.

Record Current Location: Used to record current stage position.

Shift All Locations: Moves all listed locations by a defined distance.

Move to Selected/Next/Previous: Moves stage to indicated location.

Go To Position: Drop-down list allowing user to select desired position for move stage.

Load XY Stage Locations: Used to load previously saved stage positions.

Save XY Stage Locations: Used to save stage positions. This is necessary to choose desired location in a T-series experiment.

Step Size: Allows user to define distance to move stage per arrow-click.

Set Origin (0, 0): Sets current x-y location as the origin.

Go To Origin: Returns stage to x-y position defined as origin.

7.2.11 Misc

Under the Misc tab is the label function, which gives the user the flexibility to define and save a set of operational parameters for imaging control including laser power, PMT settings, channels and pixel dwell time. It is also possible to set up label groups for more complicated or multi-laser applications.

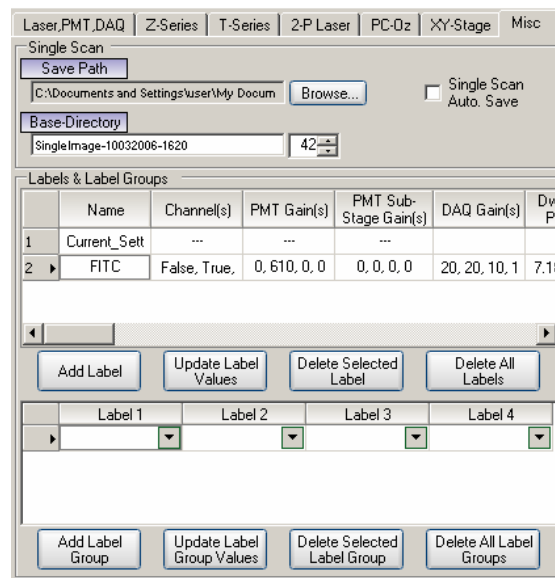


Figure 7.17: Misc tab.

To create and use a label:

- Press the '**Add Label**' button. A row named "Label-001" with current settings will be created.
- To change the name of the label, double-click on the box with "Label-001" in it and rename as desired.
- To change any of the settings associated with the label, first adjust the setting in the appropriate place (for example, an increase in laser power would be done in the 'Laser,DAQ,PMT' tab. It is then necessary to click the '**Update Label Values**' button.
- Selection of a specific label is done by clicking on a label listed under the 'Label Select' drop-down menu.

7.2.12 Scan Information

At the bottom of the PrairieView Main Window are some boxes containing scanline and frame rate information as shown in Figure 7.17. It also gives current stage location information,

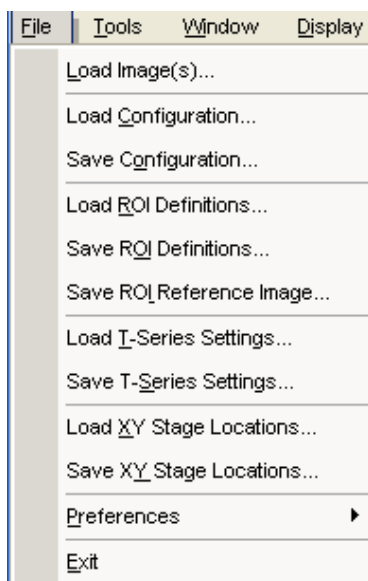
Scanline Period [ms]: 4.352	Frame Period [ms]: 2228.224	Framerate [fps]: 0.449
X = -134.30	Y = 151.88	Z = 78.30
Acquisition took 2.61 [s].		

Figure 7.18: Scan information.

7.3 *PrairieView* Toolbar Options

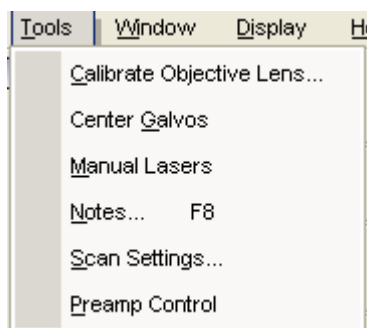
The *PrairieView* toolbar contains many resources and settings not shown on the main window. They are described briefly below.

7.3.1 File



- **Load Images.** Loads saved image files.
- **Load Configuration.** Loads system configuration file. Resets *PrairieView* control settings of the laser, PMT, scan rate, scan rotation, etc. to values previously saved.
- **Save Configuration.** Saves system configuration file. Saves *PrairieView* control settings of the laser, PMT, scan rate, scan rotation, etc. to values previously saved.
- **Load ROI Definitions.** Loads previously saved region of interest settings.
- **Save ROI Definitions.** Saves defined region of interest settings.
- **Save ROI Reference Image.** Saves reference image with saved ROIs marked on it.
- **Load/Save T-series Settings.** Loads previously saved T-series or saves one that has just been set up.
- **Load/Save XY Stage Locations.** Loads previously saved XY stage locations or saves one that has just been set up.
- **Preferences.** Allows user to select options for post-acquisition settings and t- and Z-series setting.
- **Exit.** Stops *PrairieView* and closes the program.

7.3.2 Tools

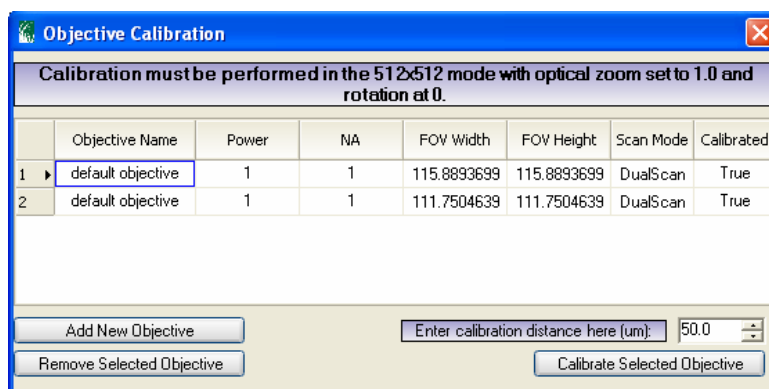


- **Calibrate Objective Lens.** See below.
- **Center Galvos.** Resets galvos to center position.
- **Manual Lasers.** A special function which may or may not apply to your system.
- **Notes.** Open window for user notes. Will be saved with all subsequent images.
- **Scan Settings.** If this option is active on your system DO NOT change any settings except under the direction of Prairie personnel. These settings are not intended for operator use.
- **Preamp Control.** Function not available at this time.

7.3.2.1 Calibrate Objective Lens

Each objective lens used on the Ultima must be calibrated. The image size must be set to 512 x 512 and 1x zoom. A calibration slide is needed for this process. The calibration steps are as follows:

- Move the objective lens to be calibrated into position on the microscope.
- Focus on a slide containing an object of known width (a calibration slide is recommended for this purpose).
- Under the 'tools' menu, select '**Calibrate Objective Lens**'.
- The following window will appear:



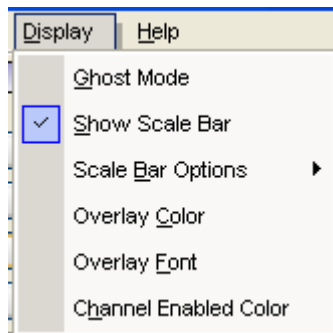
- Select '**Add New Objective**'.
- Fill in the information for objective name, power, and NA.
- Enter the calibration distance (obtained by drawing a *horizontal* line from edge to edge on the calibration object).

- Click on '**Calibrate Selected Objective**'.
- The FOV Width and Height field will now contain values and the Calibrated field should say 'True'
- Close this window.
- All calibrated objectives shown in this window will also appear under 'Objective Lens' the PrairieView Main Window.

7.3.3 Window

Opens a new Image Window. Functions the same as the 'New Window' button.

7.3.4 Display



- **Ghost.** This utility that causes the Main Control Window to appear translucent. It may be useful when combined with a dark desktop background color and operating the system in a darkened room.
- **Show Scale Bar.** Places a scale bar on the Image Window.
- **Scale Bar Options.** Allows user to select position of scale bar in Image Window as well as units of scale bar.
- **Overlay Color/Font.** Allows user to select overlay color/font in Image Window.

7.3.5 Help

Under the 'Help' option, the user will find supporting material regarding the Ultima, including a copy of the user manual and software revision notes.

7.4 Image Window

Images acquired on the Ultima are displayed in PrairieView Image Windows. It is possible to have multiple windows open at once. Each Image Window is identified by a label in the banner section with a number e.g., "Image Window – 1". All open windows will be updated when the 'Live Image' or 'Single Scan' buttons are pressed.

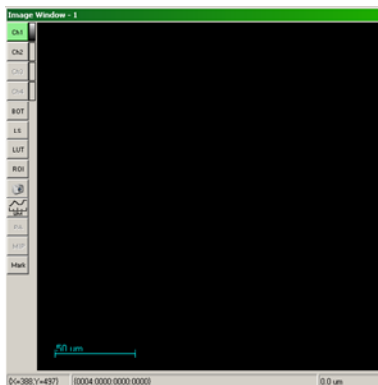


Figure 7.19: The Image Window.

Note: Some consideration should be paid to the number and size of active Image Windows that are opened, as the system may become sluggish when attempting to update too many windows simultaneously.

Located on the left side of the Image Window are buttons for the following controls (with more detailed descriptions following):



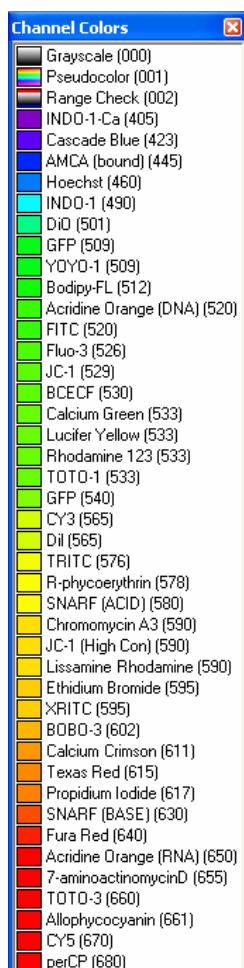
- **Ch1/Ch2/Ch3/Ch4.** The channel buttons indicate which input channel is being used to collect data for that image window.
- **BOT (Brightness-Over-Time).** Used for measurement in average pixel intensity of user-defined regions to be monitored over time.
- **LS (Line Scan).** Used for definition of line scan and length of scan acquisition.
- **LUT (Look Up Table).** Used to adjust the display intensity scale for an image.
- **ROI (Region of Interest).** Used to define a region of interest from which to obtain an image.
- **Snap (Camera Icon).** Saves the current image displayed in an Image Window.
- **µm (Line Profile Measurement).** Used to calculate the FWHM..

- **PA (Photoactivation).** Used to apply masks to specific slices in a Z-series.
- **MIP (Maximum Intensity Projection).** Displays the maximum intensity profile for a position in the z-plane at a user-selected x-y coordinate point.
- **MP (Mark Points).** Allows the user to perform a marked points experiment in **PrairieView**.

Note: *To use the BOT, LS, LUT, ROI and MP functions, Live Scan must be stopped and a single scan acquired before selection these functions. To use the PA function, a Z-series must be defined.*

7.4.1 Selecting an Input Channel (Ch1/Ch2/Ch3/Ch4)

The top four buttons on the left-hand side of the Image Window indicate which input channel is to be used to collect data for that window. These input channels are generally PMTs, but can also be from another detection device (such as a Dodt detector). Only four input channels are available at any given time. By convention, 'Ch1' represents PMT 1 on the *PrairieView* Main Window, 'Ch2' represent PMT 2, etc.



To turn on the channel, simply click on the channel button.

To select a display color for a channel, right click on that channel button and a dropdown menu with choices will appear. It is important to note that if a solid color is selected for each input channel, then multiple channels may be selected for simultaneous display within a single Image Window. Additionally, the same input channel may be displayed in multiple windows if desired.

Please note that the gray scale and pseudo-color options may not be combined with multiple channels in one Image Window.

7.4.2 'BOT' – Brightness Over Time

Pressing the '**BOT**' button located on the left hand side of an Image Window enables the measurement of pixel intensity over time.

In the Brightness Over Time window, press '**Add New**' to add regions for BOT data acquisition. Using the mouse, draw boxes in the regions of interest (up to 32). Press '**Start**' to begin acquisition. The graph in the Brightness Over Time window displays the average intensity over time of the outlined regions.

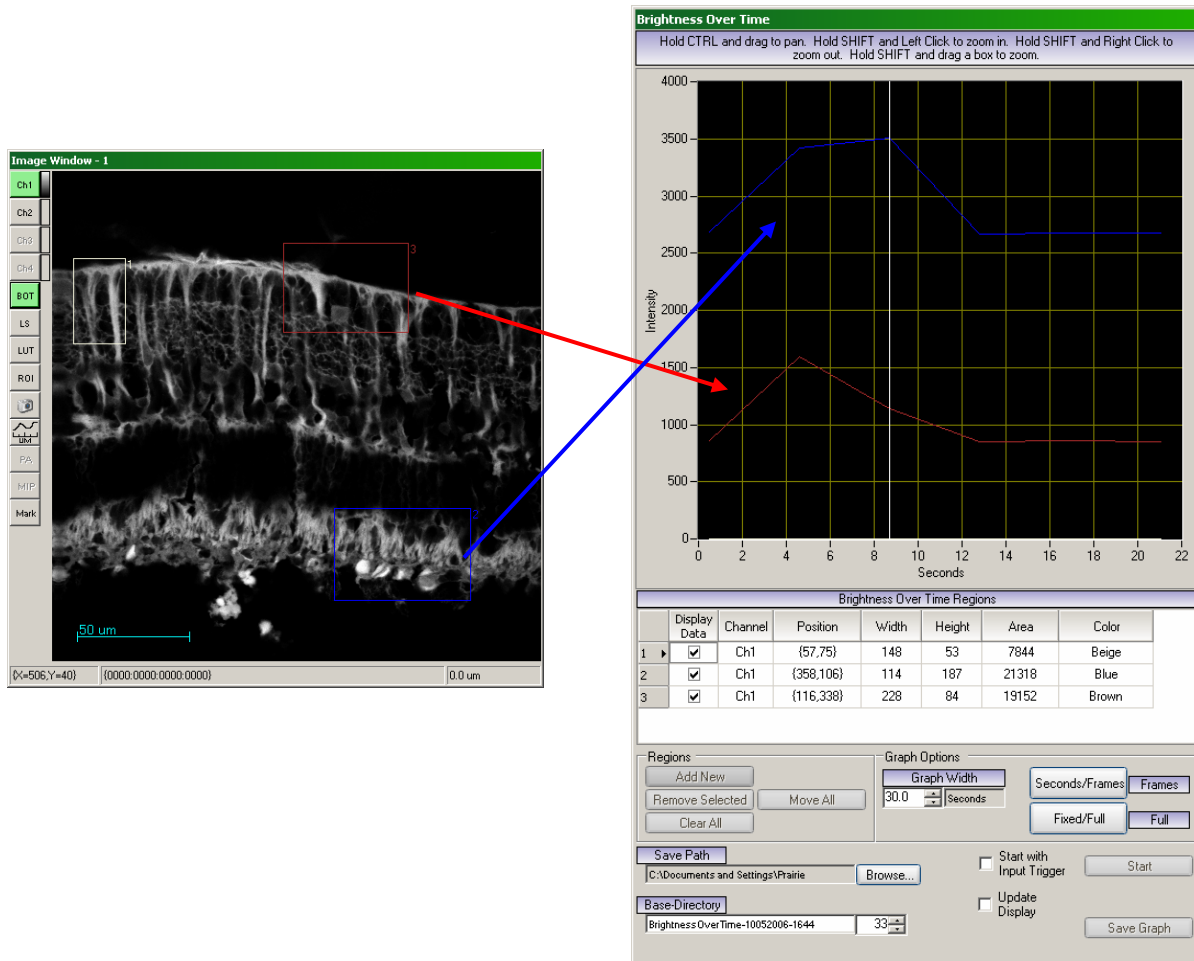


Figure 7.20: Brightness Over Time windows.

7.4.3 'LS' – Line Scan

Pressing the '**LS**' button located on the left hand side of an Image Window enables the line scan definition tool.

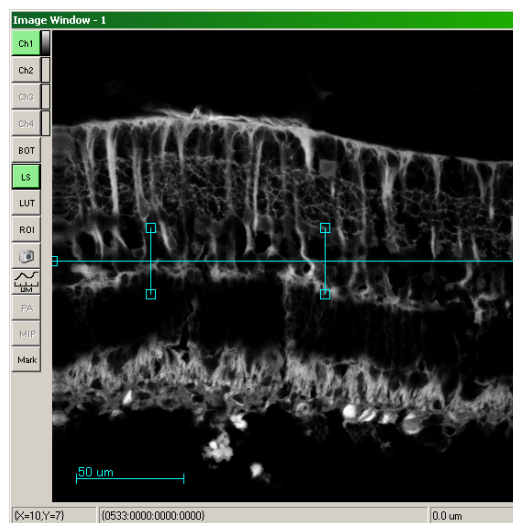


Figure 7.21: Line Scan Definition tool.

Using the mouse, click and hold on either of the outside blue boxes to position the line scan location vertically. Use the inner pair of blue boxes to select the lateral range to scan. The laser beam will **ONLY** scan the selected locations, thereby reducing the amount of photo damage to the specimen.

A Linescan Control window appears when the '**LS**' button is pressed. This window is used to define the number of lines to acquire in succession, the number of repetitions to acquire those lines, and the period for the acquisition. It is also used to indicate any trigger controls and allows the user to select a free draw option, by checking the appropriate box. Click on '**Start Linescan**' to begin the acquisition. The acquired data will be displayed below 'Base Directory'. Once the line scan is complete, this mode can be exited by again pressing the '**LS**' button on the Image Window.

It is possible to look at acquired line scan data by opening the Linescan Viewer program located on the Ultima computer desktop. Under 'File', find and open the line scan data of interest. An image file will open, rotated 90 degrees as shown in Figure 7.21 (so that the x-axis is defined as time).

In the Linescan Viewer, it is possible to look at the line profile of the image data (pixel dwell rate) by selecting a position of interest along the line. If electrophysiological data (in samples/second) has also been acquired, it is possible to overlay the two sets of data to see how the image data and the electrical data correlate in time.

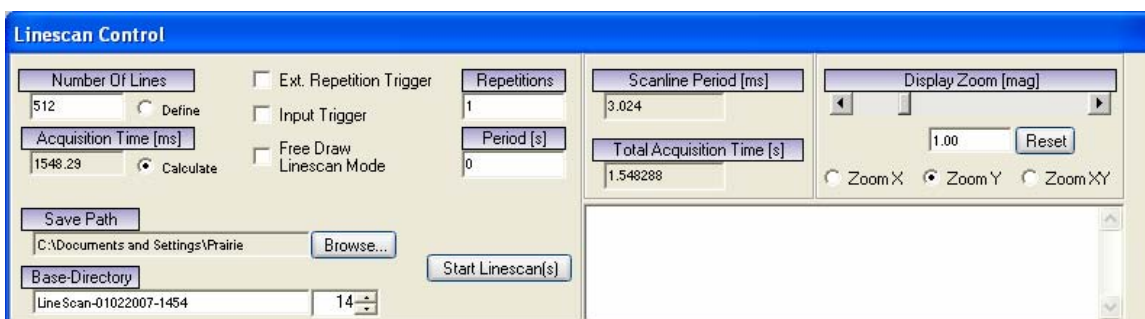


Figure 7.22: Linescan Control.

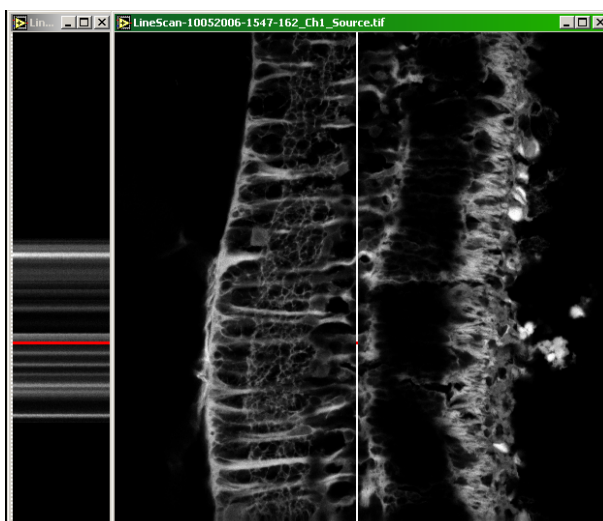


Figure 7.23: Line Scan Data display.

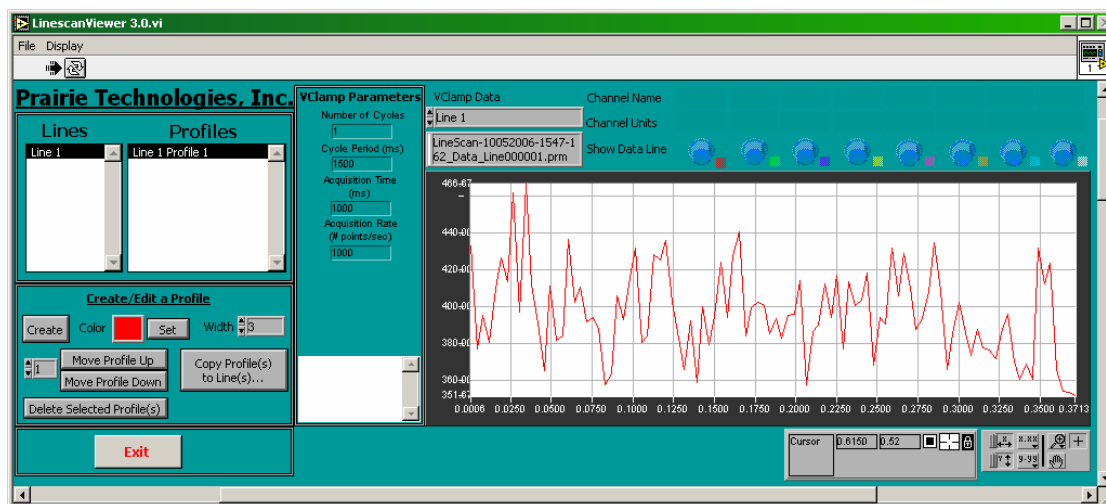


Figure 7.24: Linescan Viewer.

NOTE: If the TriggerSync program is running the operator is prompted to enter a file name for the line scan image and electrical data. All the data will be saved once the line scan is completed.

NOTE: *If the TriggerSync program is running, Line Scan acquisition will automatically synchronize with the experiment that has been defined in TriggerSync. A coordinated recording will be made that synchronizes the electrical recording from TriggerSync with the image data from the PMTs. Use the 'Linescan Viewer' program located on the Windows desktop to view both the line scan image and electrical data that has been collected in this manner.*

7.4.4 'LUT' – Look-Up Table

A Look Up Table (LUT) is the function that is used to color the display of the data to be displayed on the computer screen.

The images from the Ultima are digitized to 12 bits, which means that the input channel *data* intensity scale ranges from 0 (no signal) to 4095 (saturated signal). In a black and white LUT, values of 0 are usually represented as pure black and values of 4095 are usually represented as pure white. Since the computer only has 256 grey levels, a function or LUT is used to define the *display* intensity scale. If these 256 display grey levels are used to display the full range of 4096 intensity levels then each display grey level is equal to 16 PMT data intensity levels.

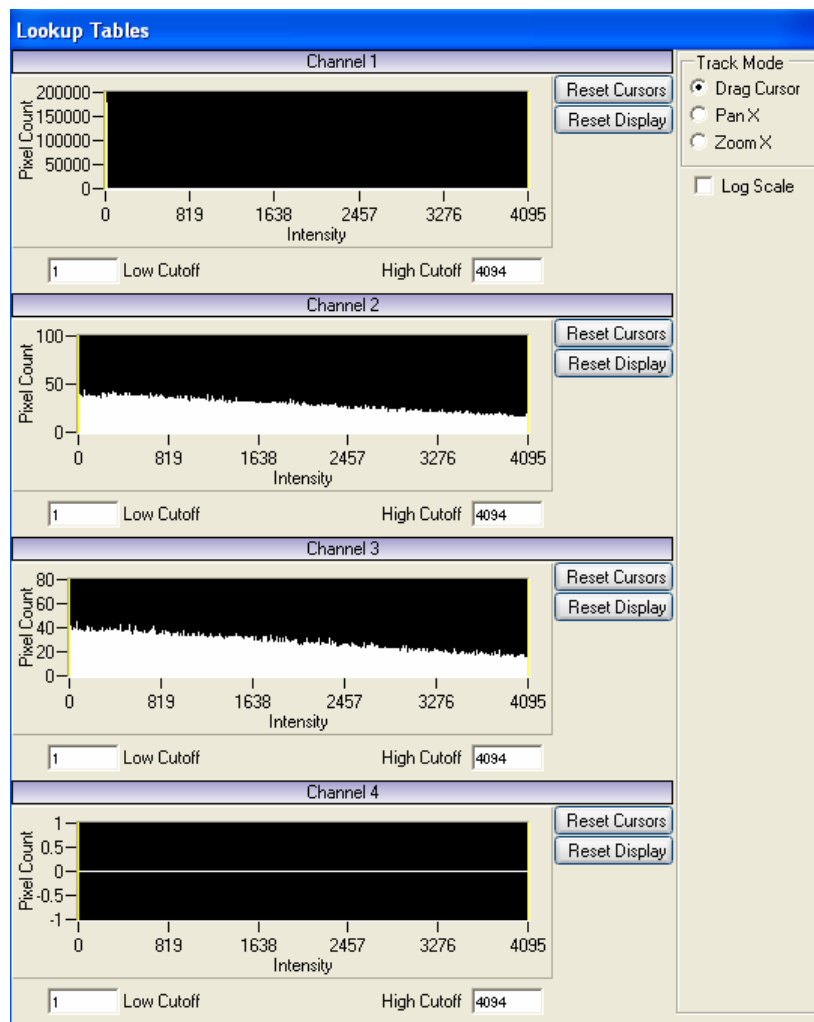


Figure 7.25: Lookup Table.

Since fluorescent images very often contain a large number of dark pixels, it is sometimes desirable to adjust the range of the LUT to exclude some of the dark pixels, thereby allowing the LUT to display smaller changes in the data intensity scale.

There are three options for looking at intensity data in the LUT. 'Drag Cursor' allows the mouse to be used to adjust the high and low ranges of the LUT used for each channel by dragging the yellow lines. The Low and High Cutoffs give the range defined by the yellow lines. 'Pan X' and 'Zoom X' let the user moving the graph to more carefully ascertain the pixel intensity characteristics of the input channel data. 'Log Scale' allows meaningful information about the intensity scale to be viewed for images with a large dynamic range.

7.4.5 'ROI' – Region of Interest

It is often useful to scan only a selected region of the field of view to optimize image speed. Using the Region of Interest (ROI) tool, it is possible to define a small portion of the sample to be imaged, which increases the scan rate up to hundreds of frames per second. When this button is pressed, the cursor is used to define a rectangular area in which to limit scanning.

To define ROIs after clicking the '**ROI**' button, place the cursor at one corner of the area of interest. Click and drag the mouse, creating a rectangular ROI. Click again to release.

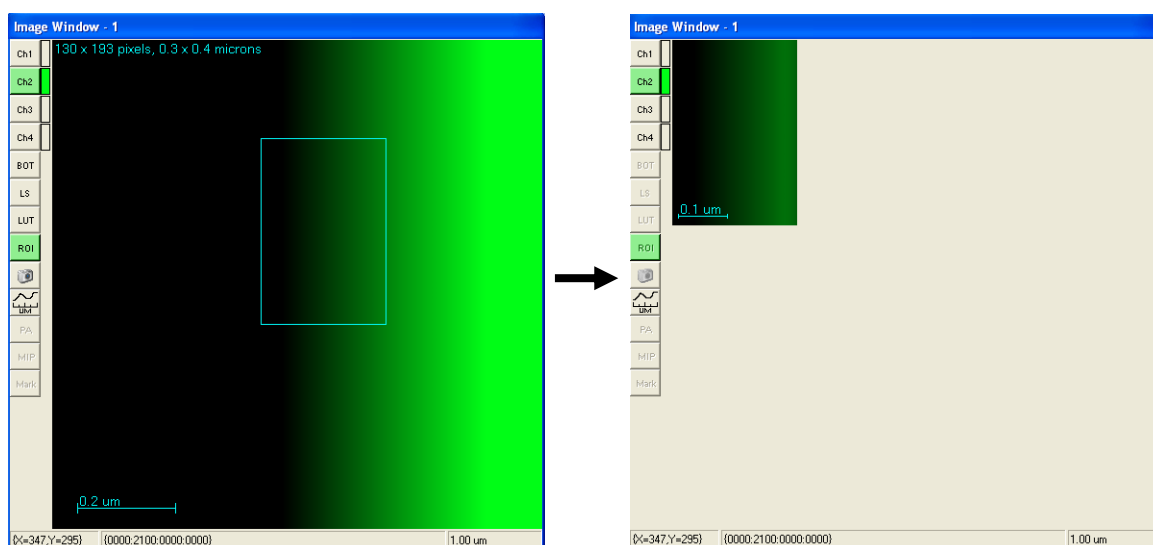


Figure 7.26: Define an ROI and ROI image.

The Image Window will automatically resize itself. Press either '**Live Scan**' or '**Single Scan**' to refresh the display to reflect the ROI. The laser beam is **ONLY** scanned over this portion of the specimen.

When '**LiveScan**' is 'stopped', it is possible to right-click on the '**ROI**' button to open a 'Save' window to name and save ROIs.

7.4.6 Snap Tool

The Snap button is used to save the current image displayed in an Image Window. The image will be saved to the filename and path specified in the PrairieView Main Window.

If any overlays are present on the image, the exact displayed view (including display zoom) and overlays will be saved as an 8-bit TIFF file. If there are no overlays present, the displayed image will be saved as if it were a single image acquisition (a 16-bit metadata file).

7.4.8 'μm' – Line Profile Measurement

The 'μm' tool displays a plot of intensity along a user-defined line on each of the active channels.

When the 'μm' button is clicked, a line appears on the image window and a 'Line Profile' window opens. By dragging the ends of the line in the image window, it can be positioned across the area of interest. By moving the white and dark blue marks along this line (in either window), the area of interest can be more sharply defined for FWHM calculations. To calculate the FWHM of the area between these cursors, choose the desired channel under '**Select Ch**' and click '**FWHM**'

When the 'MIP' mode is active (See Section [7.4.9](#)), the Z-Mode option on the Line Profile window can be used to acquire a profile or calculate FWHM along the z-axis at a point in X-Y (as defined in MIP).

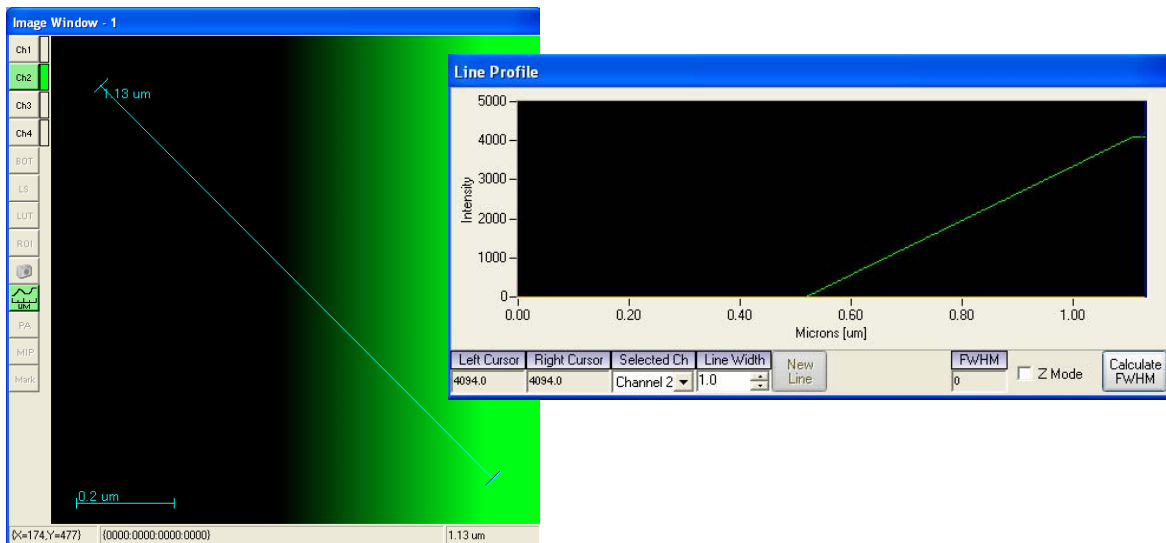


Figure 7.28: 'μm' (Line Profile Measurement) windows.

7.4.9 'MIP' - Maximum Intensity Projection

The 'MIP' tool displays the maximum intensity profile for a position in the z-plane at a user-selected x-y coordinate point.

To use the MIP tool, a Z-series must first be acquired, as described in Section [7.2.8](#). While still in the playback window, click the '**MIP**' button. This places a crosshairs cursor on the image and opens up a sidebar window to the right of (the y-z projection) and below the image window (the x-z projection). By moving the cursor to the x-y coordinate of interest, the user can observe changes in intensity in z. As the cursor is moved along the x-axis, intensity changes in the y-z plane are observed, while as the cursor is moved along the y-axis, intensity changes in the x-z plane are observed.

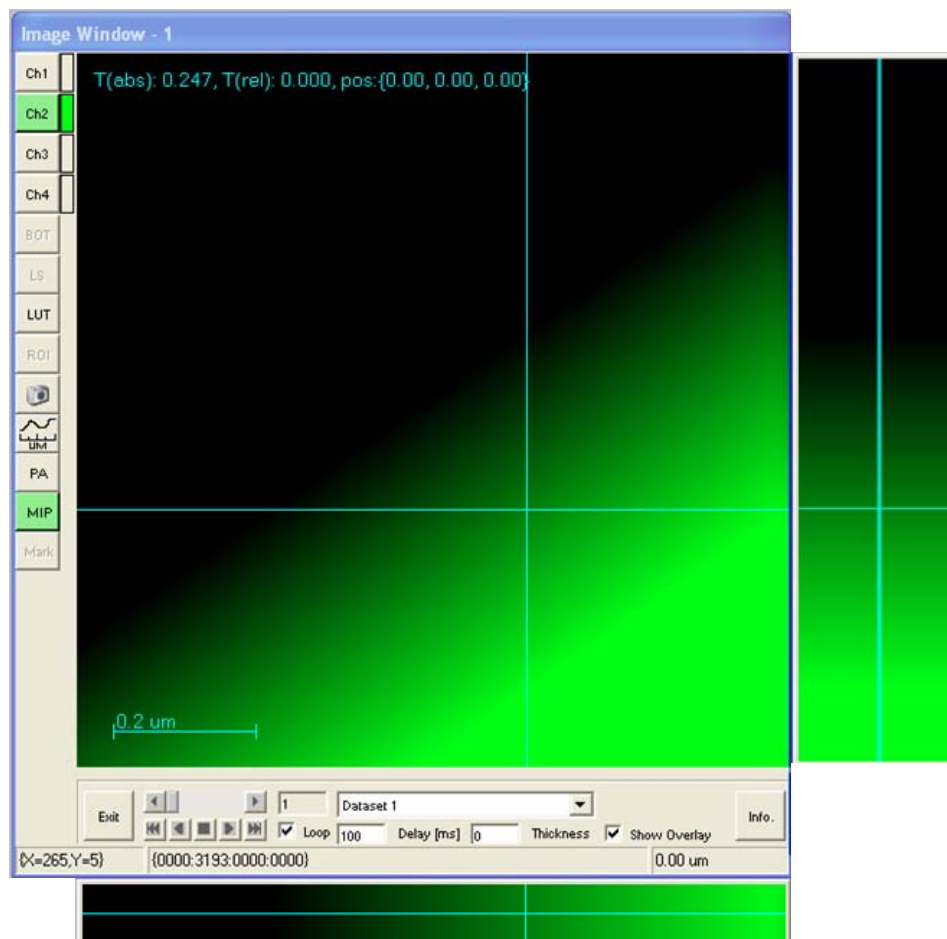


Figure 7.29: MIP (Maximum Intensity Projection) window.

7.4.10 'MP' – Marked Points

The 'Mark Points' dialog is used to mark locations of points for photoactivation or uncaging experiments. These points can be single or in a user-defined line or grid.

Note: Although the Mark Points function can be performed in a *PrairieView* window, *TriggerSync* must be open and running in order to use this function.

When the '**Mark**' button is clicked, a box appears on the image in the active Image Window and a second, "Mark Points", window appears (as shown in Figure 7.28). To select points, click on the appropriate option in the Mark Points window and follow the steps described below. Because this window is an interface between the *PrairieView* and *TriggerSync* programs, it is necessary to set the Mark Points parameters using a Mark Points Wizard, which is accessed by clicking on the '**Configure**' button.

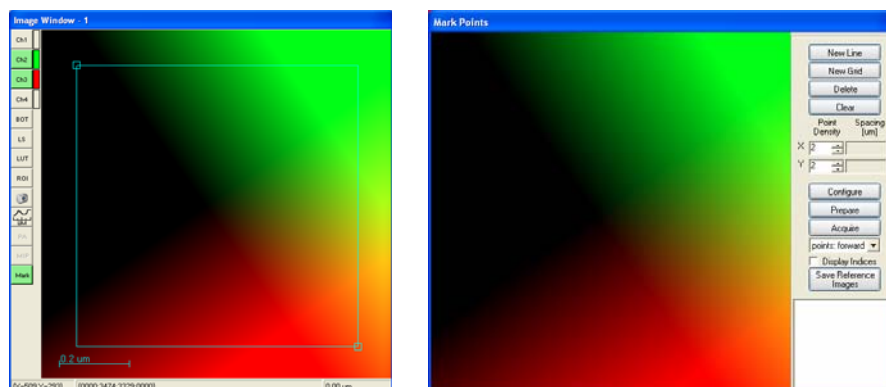


Figure 7.30: Image Window and "Mark Points" window.

To mark a point:

- Click on '**New Point**'.
- Select the point of interest in the dialog box by clicking (will highlight line).
- Move point to desired location on image.
- Click '**Configure**' to set Mark Points parameters.
- Click '**Save Reference Image**'.
- Click '**Acquire**' to mark points.

To mark a line of points:

- Click on **'New Line'**.
- Select the line of interest in the dialog box by clicking (will highlight line).
- Enter number of points in X "Point Density" field.
- Move line to desired location on image.
- Click **'Configure'** to set Mark Points parameters.
- Click **'Save Reference Image'**.
- Click **'Acquire'** to mark points.

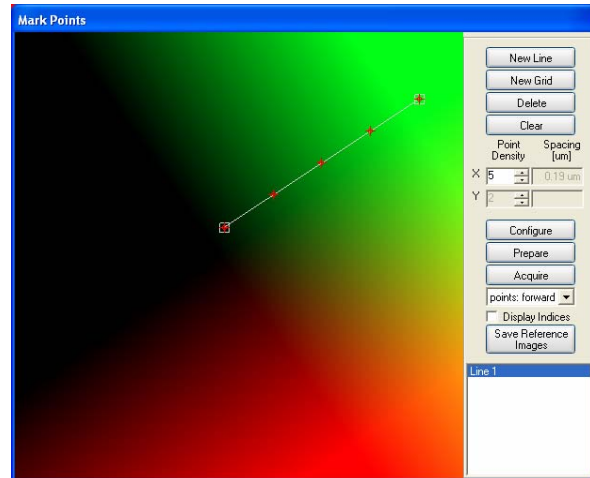


Figure 7.31: Mark Points: line.

To mark a grid of points:

- Click on **'New Grid'**.
- Select the grid of interest in the dialog box by clicking (will highlight line).
- Enter number of points in X and Y "Point Density" fields.
- Move grid to desired location on image by dragging center point.
- Resize and rotate grid by dragging corners.
- Click **'Configure'** to set Mark Points parameters.
- Click **'Save Reference Image'**.
- Click **'Acquire'** to mark points.

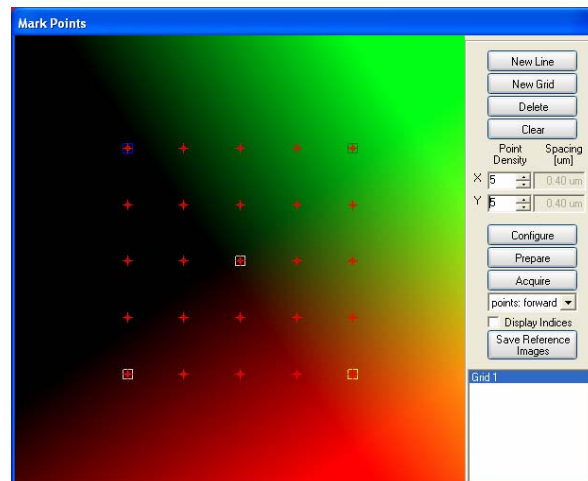


Figure 7.32: Mark Points: grid.

Note: Information on marking points in TriggerSync can be found in Section [8.5](#).

8 TriggerSync Software

TriggerSync is the Prairie application that integrates electrophysiology recording with image collection. From *TriggerSync* the user can define output signals for stimulation of a sample preparation or to control external equipment such as shutters or pumps. *TriggerSync* also allows the user to collect image and line scan data and to use this information in the selection of discrete locations where photolysis is to be performed.

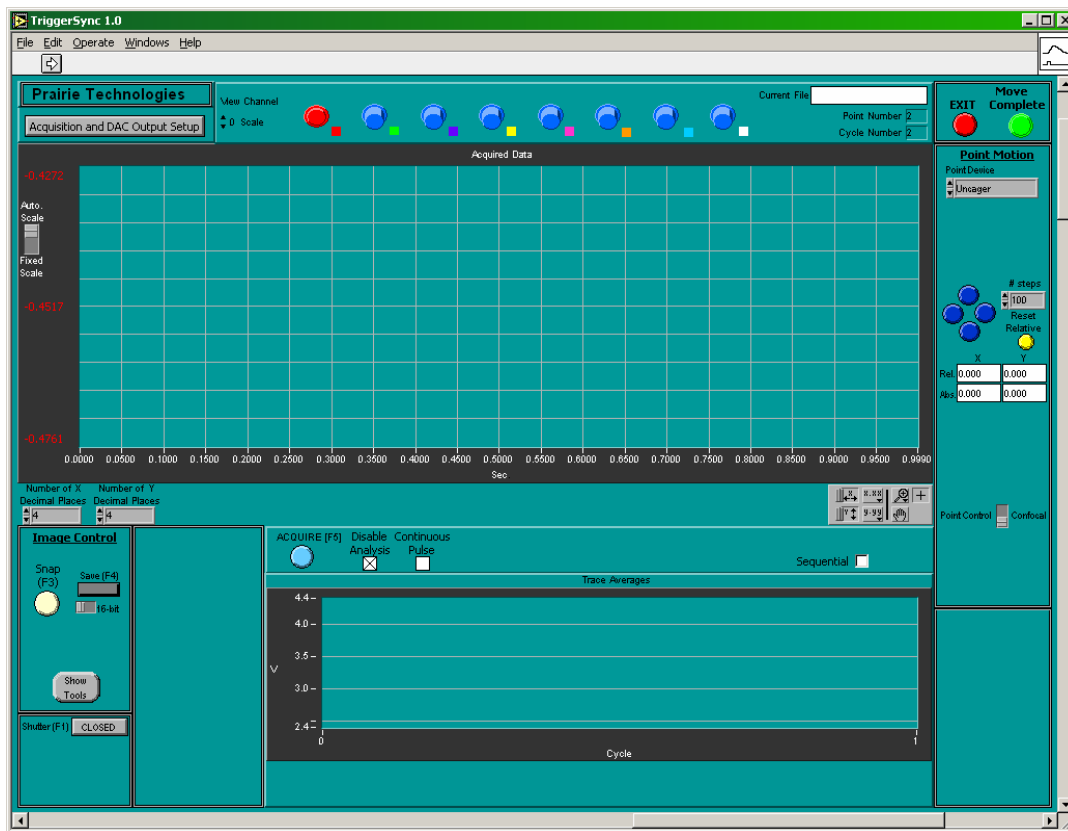


Figure 8.1: Main *TriggerSync* window.

8.1 Main Operational Modes

The *TriggerSync* program operates in two modes. When the application is first started, by default it is in acquisition mode. While in this mode, the user may define various acquisition parameters such as input and output settings, calibrate the pointing device, and mark points. After performing an acquisition, the program is in the post-acquisition mode. While in this mode, the user can not change acquisition parameters but can manually scroll through the acquired data, performing basic viewing functions such as changing the vertical and horizontal scaling. It is important to remember that by default, as soon as an

acquisition is completed, the *TriggerSync* application will go into the 'Analyze Acquired Data: On' state.

The operator may manually switch between these two modes at any time by clicking on the '**Analysis**' menu option and then clicking on the '**Analyze Acquired Data (Ctrl+A)**' option. This menu option will show a checkmark in front of it when the Analyze Acquired Data mode is on. When no checkmark is visible, then the Analyze Acquired Data mode is off.

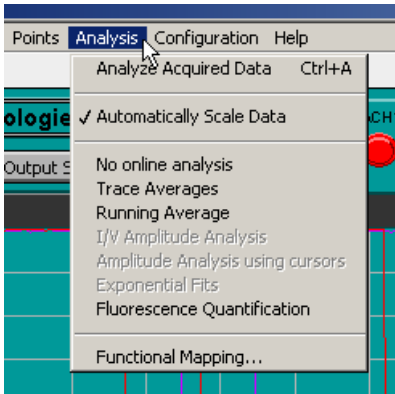


Figure 8.2: Analyze Acquired Data mode.

There is an additional control that overrides the Analyze Acquired Mode menu option. To the immediate right of the '**Acquire**' button is a checkbox control labeled '**Disable Analysis**'. When this button is checked (enabled), it will prevent the operator from turning the Analyze Acquired Data mode on.

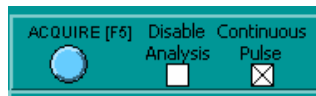


Figure 8.3: *TriggerSync* 'Acquire'.

This override control is present because some operators wish to have full access to all of the input and/or output controls as soon as the current acquisition is finished without having to first turn the Analyze Acquired Data mode off.

8.1.1 Analyze Acquired Data: Off

As mentioned, this is the default mode of operation when the *TriggerSync* application is first started. If the '**Disable Analysis**' control is checked, then this is the only mode of operation for *TriggerSync* until the '**Disable Analysis**' control is unchecked.

This is the operational mode that *TriggerSync* must be in for the operator to set the various acquisition parameters, calibrate the pointing device, and create a list of marked points.

8.1.2 Analyze Acquired Data: On

This mode is intended for post-acquisition evaluation of the data. This includes analysis of the most recently acquired data as well as analysis of previously acquired and saved data. When the Analyze Acquired Data mode is on, several menu options are disabled (as shown in Figure 4). Also, most of the controls on the main *TriggerSync* window will also not operate (although they may not be disabled like the menu options). In addition, when Analyze Acquired Data mode is on, some additional controls will become visible in the main *TriggerSync* window.

8.1.2.1 View Channel Sensitivity

At the top center of the window, just left of the buttons that are used to select the desired input channels is a control labeled '**View Channel Sensitivity**'. This control specifies which input channel's vertical scale (y-axis) values will be displayed along the left hand side of the graph area. The values for the channels minimum, maximum, and middle will be displayed in the same color as that specified for the selected input channel.

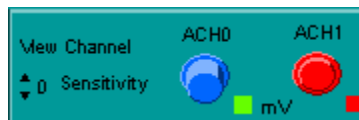


Figure 8.4: 'View Channel Sensitivity'.

8.1.2.2 'Change Scale' and 'Auto Scale'

Along the left hand side of the graph area will also appear two button controls '**Change Scale**' and '**Auto Scale**'.

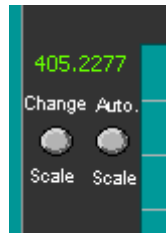


Figure 8.5: Change Scale & Auto Scale.

When the '**Change Scale**' button is pressed it will open the ADC Configuration dialog. This dialog will allow the operator to specify whether each acquired data channel should be automatically scaled or whether the operator wishes to fix the minimum and maximum values that the data should be scaled against. For a complete description, see the 'ADC Configuration' Section 8.3.2 later in this document.

When the **'Auto Scale'** button is pressed, each of the acquired data channels will be automatically scaled. When automatically scaled, the data in each channel is analyzed to determine the minimum and maximum values and these will be the values that the data in each channel will be scaled against.

8.1.2.3 'Sequential File'

Below the graph window and to the right of the **'Acquire (F5)'** button is a box labeled **'Sequential'**. When this box is checked, a file selection dialog will be presented to the operator allowing him/her to specify the directory and file name to be used for saving the current cycle data in a format readable by a spreadsheet program such as Microsoft Excel.

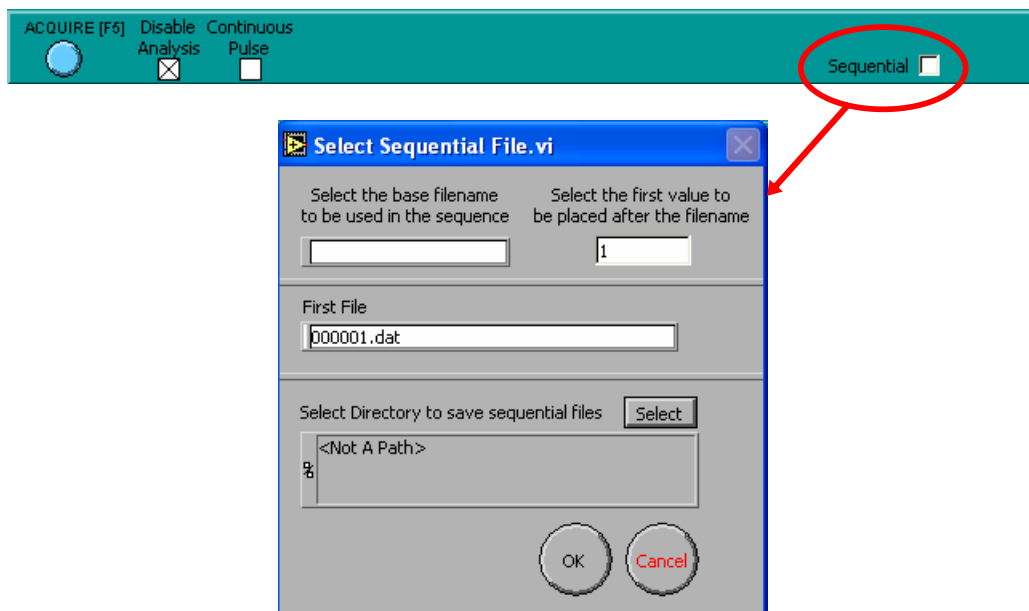


Figure 8.6: Sequential File.

8.1.2.4 'Save Experiment'

A little further to the right is a button labeled **'Save Experiment'**. When this button is pressed, the operator will first be prompted as to whether the experiment should be saved in *'TriggerSync'* format (this includes all operating parameters as well as the data) or to *'Export'* format (this includes only the data), which is a format readable, by a spreadsheet program such as Microsoft Excel.

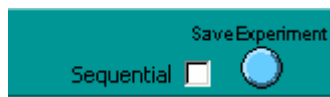


Figure 8.7: Save Experiment.

8.2 Acquisition and DAC Output Setup

In the upper left hand corner of the main window of *TriggerSync* is a button labeled 'Acquisition and DAC Output Setup'. Pressing this button will bring up a dialog that the operator uses to control various input and output parameters to be used during a *TriggerSync* acquisition. While this dialog is open, the operator may not adjust any parameters in *TriggerSync* or any other *TriggerSync* dialog that are not part of this dialog.



When this dialog is opened, the Mark Points dialog will automatically be closed and the 'Acquisition and DAC Output Setup' dialog opens.

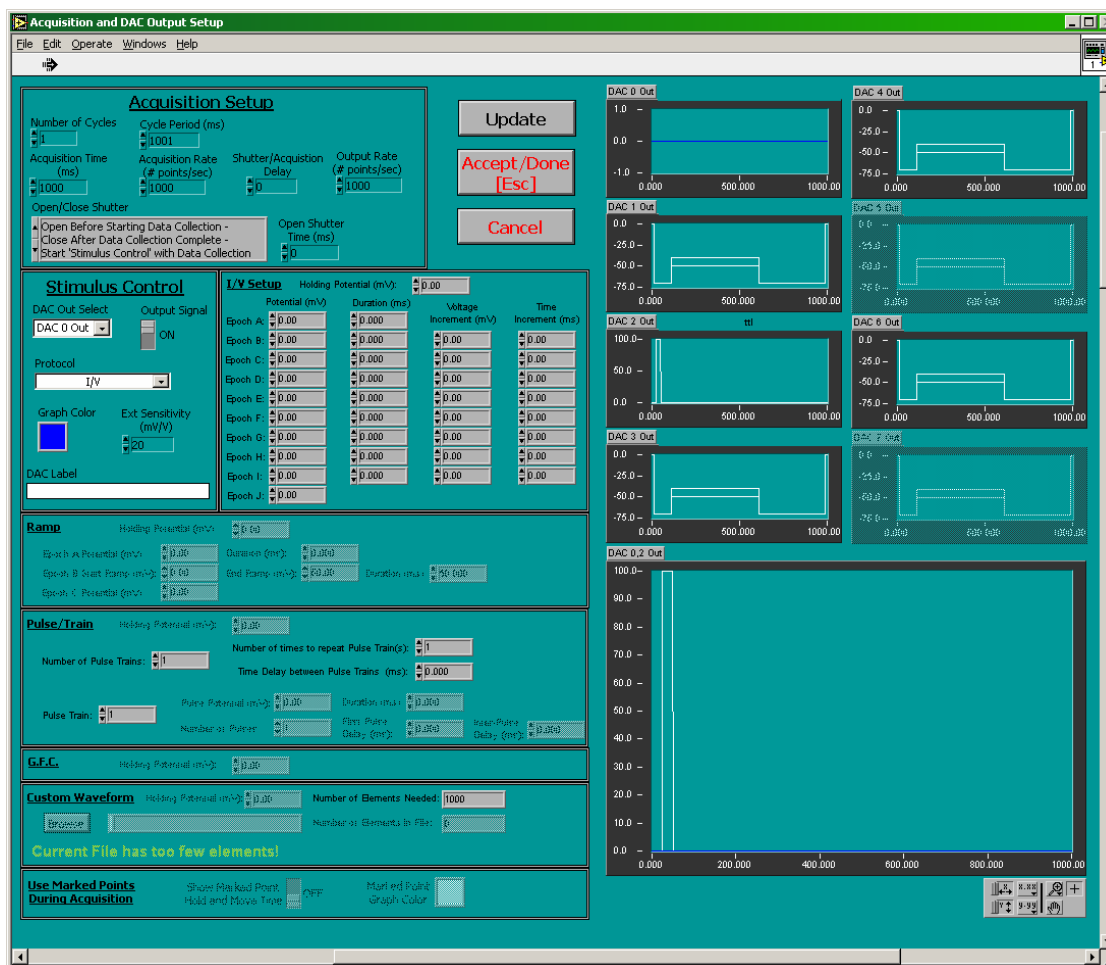


Figure 8.8: Acquisition and DAC Output Setup.

8.2.1 Acquisition Setup

Acquisition Setup

Number of Cycles	Cycle Period (ms)		
1	351		
Acquisition Time (ms)	Acquisition Rate (# points/sec)	Shutter/Acquisition Delay	Output Rate (# points/sec)
350	1000	0	2000
Open/Close Shutter			Open Shutter Time (ms)
<input checked="" type="checkbox"/> Open Before Starting Data Collection - Close After Data Collection Complete - Start 'Stimulus Control' with Data Collection			0

Figure 8.9: Acquisition Setup.

Number of Cycles: 'Number of Cycles' indicates the number of acquisitions to perform when the 'Acquire' button is pressed.

Cycle Period: 'Cycle Period' specifies the amount of time (in milliseconds) for each cycle. This value must be at least 1 ms longer than the 'Acquisition Time'.

Acquisition Time: 'Acquisition Time' specifies the amount of time that data should be acquired when the 'Acquire' button is pressed.

Acquisition Rate: 'Acquisition Rate' specifies the rate at which the inputs should be sampled during the acquisition. To avoid aliasing issues, the 'Acquisition Rate' should be at least twice the anticipated rate of signal change the operator wishes to detect.

Shutter / Acquisition Delay: 'Shutter/Acquisition Delay' indicates the amount of time to wait after opening the shutter before starting the acquisition. This parameter could be of use when working with a slow mechanical shutter where the operator wants to guarantee that the shutter is fully opened before collecting the electrical data.

Output Rate: 'Output Rate' controls the step size of the output DAC signals during the acquisition. Essentially, by having a larger 'Output Rate' value, there is finer resolution in the voltage to time relationship on the output DAC signals.

Open / Close Shutter: 'Open/Close Shutter' specifies when the shutter should be opened and closed relative to the data acquisition. There are three possible options:

A) *Open Before Starting Data Collection - Close After Data Collection Complete - Start 'Stimulus Control' with Data Collection,*

B) Open After Starting Data Collection - Close After 'Open Shutter Time' milliseconds - Start 'Stimulus Control' with Data Collection, and

C) Open After Starting Data Collection - Close After 'Open Shutter Time' milliseconds - Start 'Stimulus Control' after Shutter is Opened.

In option 'A' the shutter will be opened first, then the acquisition and stimulus control (output signals) will happen and then after the acquisition and stimulus control has completed, the shutter will be closed.

In option 'B', first the acquisition and stimulus control will start, then the shutter will be opened, the shutter will remain open for the amount of time specified by 'Open Shutter Time', and then close. The acquisition time is determined by the setting of 'Acquisition Time'.

In option 'C', first the acquisition will start, then the shutter will be opened and the stimulus control will begin, the shutter will remain open for the amount of time specified by 'Open Shutter Time', and then close. The acquisition time is determined by the setting of 'Acquisition Time'.

Open Shutter Time: 'Open Shutter Time' specifies the value for the amount of time in milliseconds that the shutter should be opened based upon the setting for the 'Open/Close Shutter' control.

8.2.2 Stimulus Control

Depending upon the system configuration, the operator has the ability to program a maximum of from 2 to 8 analog output signals. Along the left hand side of the dialog there is a section labeled 'Stimulus Control'.



Figure 8.10: Stimulus Control.

The entire right half of the dialog is used to provide graphical display of each of the possible DAC output waveforms independently and then the bottom most graph (the largest) displays the DAC output waveforms overlaid on one another for all of the DAC outputs that have been turned on. The label on this composite graph includes the names of the analog outputs that are currently turned on.

In the top center of the dialog are two buttons 'Update' and 'Accept/Done'. As the operator makes changes to the various fields in this dialog, the graphical displays will only be updated when the 'Update' button is pressed. After all desired changes have been made; press the 'Accept/Done' button to close the dialog.

DAC Out Select: To program an analog output first select the desired output using the 'DAC Out Select' control. The labels in this control correspond to the labels on the BNC connectors on the BNC-2110 box (or BNC-2090 box if no BNC-2110 is present).

Output Signal: After selecting the desired output channel, use the 'Output Signal' control to turn this analog output on/off when performing an acquisition.

Protocol: 'Protocol' control allows the operator to select a pattern, or Protocol for the selected analog output. The operator may select from the following Protocols: *I/V*, *Ramp*, *Pulse/Train*, *G.F.C.*, and *Custom Waveform*. These protocols are described in more detail in Section [8.2.3](#).

Graph Color: This control allows the operator to specify the color to use for displaying the graph both in its own graph window and in the composite graph window in the lower right hand corner of the dialog.

Ext. Sensitivity: The operator may specify a separate 'Ext. Sensitivity' value (mV/V) for each analog output. The 'Ext. Sensitivity (mV/V)' control is used as a scale factor for the voltage values entered in the stimulus protocols. To determine the actual voltage being output use the following equation:

$$(1 / \text{Ext. Sensitivity}) * \text{protocol voltage} = \text{Output voltage}.$$

For example, in the G.F.C. protocol, if an 'Ext. Sensitivity' of 20 is used and the 'Holding Potential (mV)' is set to 10, the actual output voltage will be 0.5 Volts:

$$(1/20) * 10 = 0.5.$$

This scaling is applied to all voltages in all of the available protocols.

DAC Label: This control allows the operator to specify a text string to display over the individual graph window along the right hand side of the dialog. For

example, 'DAC 6 Out' is often wired to control the Pockels cell device, so the operator might want to set the 'DAC Label' for this DAC to something like 'Pockels Output'.

8.2.3 Protocols

8.2.3.1 I/V (Current / Voltage)

	Potential (mv)	Duration (ms)	Increment (mv)
Epoch A:	0.00	100.000	
Epoch B:	2.00	100.000	1.00
Epoch C:	0.00	100.000	0.00
Epoch D:	0.00	0.000	0.00
Epoch E:	0.00	0.000	0.00
Epoch F:	0.00	0.000	0.00
Epoch G:	0.00	0.000	0.00
Epoch H:	0.00	0.000	0.00
Epoch I:	0.00	0.000	0.00
Epoch J:	0.00		

Figure 8.11: I/V Setup.

When the I/V protocol is selected, the controls for the other protocols will be desensitized. The I/V protocol allows the operator to divide the acquisition time among various 'Epochs'. For each epoch the operator specifies the amount of time and the desired voltage value. In addition, for epochs B through I there is an additional field labeled 'Increment (mV)'. This field is used when running an acquisition that has more than one cycle. If the increment is any value other than 0, then after the first cycle, the voltage for the second cycle for this epoch will be incremented (or decremented if a negative value is specified) by this amount. If a third cycle is to be acquired, then the voltage for this epoch will be incremented/decremented again, and so on.

8.2.3.2 Ramp

Ramp	
Holding Potential (mv): 0.00	
Epoch A Potential (mv): 0.00	Duration (ms): 100.000
Epoch B Start Ramp (mv): 0.00	End Ramp (mv): 5.00
Epoch C Potential (mv): 0.00	Duration (ms): 200.000

Figure 8.12: Ramp.

When the Ramp protocol is selected, the controls for the other protocols will be desensitized. The Ramp protocol allows the operator to program a voltage ramp for output. Epoch A is used to specify a voltage and the time leading up to the

start of the ramp. Epoch B is used to define the actual ramp voltages (starting and ending) as well as for the length of time to spread the ramp over. Epoch C is used to specify a voltage and the time from the end of the ramp to the end of the acquisition.

8.2.3.3 Pulse/Train

Figure 8.13: Pulse/Train.

When the Pulse/Train protocol is selected the control for the other protocols will be desensitized. The Pulse/Train protocol allows the operator to define a single square voltage pulse or a train/sequence of square voltage pulses or a sequence of multiple independently defined pulses or pulse trains.

‘Number of Pulse Trains’ defines the number of independently defined pulse trains to generate. **‘Number of Times to Repeat Pulse Train(s)’** defines the number of times that the defined pulse train(s) should be repeated. **‘Time Delay between Pulse Trains (ms)’** specifies the number of milliseconds used to separate one iteration of the defined pulse train(s) from the next iteration of the defined pulse train(s).

To edit the parameters for a given pulse train, first select the pulse train to edit using the **‘Pulse Train’** control. **‘Pulse Potential’** defines the height of the pulse/train and **‘Duration’** defines the amount of time each pulse is held at the **‘Pulse Potential’**. The **‘Number of Pulses’** defines the number of pulses in the pulse train. **‘First Pulse Delay’** specifies the number of milliseconds from the start of the acquisition until the first pulse starts. **‘Inter-Pulse Delay’** indicates the number of milliseconds to separate each pulse.

8.2.3.4 G.F.C. (General Fluorescence Control)

When the G.F.C. protocol is selected the controls for the other protocols will be desensitized. This protocol simply maintains the specified **‘Holding Potential’** during the acquisition.

8.2.3.5 Custom Waveform

When the Custom Waveform protocol is selected the controls for the other protocols will be desensitized. This protocol allows the a file to be created that contains any desired sequence of voltage values. The file should be a text file that contains a column of numbers that represents the desired voltage values. The column of numbers can be in standard notations (e.g. .01003) or in scientific notation (e.g. 1.003e-2). If the file contains more than one column of numbers, only the first column shall be used. The field 'Number of Elements Needed' indicates the number of elements the operator created file should contain. This value is a function of the 'Acquisition Time' and the 'Output Rate'. For example, if the acquisition time is 500 ms (0.5 s) and the output rate is 10000 samples per second, then the number of samples needed in the file would be 5000:

$$0.5 \text{ s} \times 10,000 \text{ samples per second} = 5000 \text{ samples}$$

The field 'Number of Elements in File' indicates the number of elements that were actually in the file.

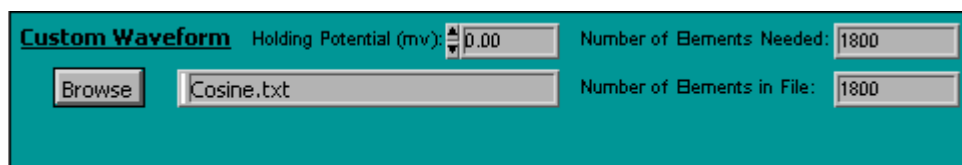


Figure 8.14: Custom Waveform.

To load a user-defined file, press the '**Browse**' button and using the pop-up file dialog select the appropriate file. If the file contains too few elements, then the remaining acquisition time will be padded with the 'Holding Potential' value. If the file contains too many elements, then the sequence will simply be truncated at the point where the acquisition time is attained.

8.2.4 Use Marked Points During Acquisition

The lower left hand corner of the dialog is labeled 'Use Marked Points During Acquisition'.

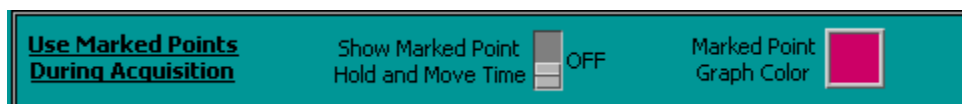


Figure 8.15: Use Marked Points During Acquisition.

If a list of 'Marked Points' has been created and the 'Use Marked Points During Acquisition' control in the 'Mark Points' dialog has turned on, then these controls would be active. If the control 'Show Marked Point Hold and Move Time' is turned on, then overlaid on the largest graph will be an additional graph in the 'Marked Point Graph Color' indicating when each marked point will occur during the acquisition.

This overlay graph will indicate when the next marked point is being moved to with a spike on the graph. To interpret the graph, when the acquisition begins the pointing device will already be positioned at the first marked point. The pointing device will stay at that location for the amount of time specified in the Mark Points dialog by adding the time for 'Marked Point Hold Time (ms)' and the 'Marked Point First Point Delay (ms)' parameters. The first spike on the graph indicates that the pointing device is moving to the second marked point. The time necessary to move the pointing device is specified in the Mark Points dialog by the 'Marked Point Move Time (ms)' parameter. The pointing device is then held at the location of the second marked point for the time specified in the Mark Points dialog by the 'Marked Point Hold Time (ms)' parameter. The second spike on the graph indicates that the pointing device is moving to the third marked point. The time necessary to move the pointing device is specified in the Mark Points dialog by the 'Marked Point Move Time (ms)' parameter. The pointing device is then held at the location of the third marked point for the time specified in the Mark Points dialog by the 'Marked Point Hold Time (ms)' parameter. This process continues for all of the marked points.

This graphical display allows the operator to configure the other output channels so that the desired event(s) will occur relative to the pointing device movement.

For example, the operator has:

1. Created a list of five marked points in the Mark Points dialog.
2. In the Mark Points dialog, the operator has set 'Use Mark Points During Acquisition' to 'On'.
3. 'Marked Point Hold Time' to 100ms.
4. 'Marked Point Move Time' to 0.1ms.
5. 'Marked Point First Point Delay' to 0.

If in the Input and DAC Out Setup dialog, the operator wishes to expose the sample for 25ms (for example by changing the Pockels output voltage from 0 to 5 volts) after moving to a new location and the data collection at that point should continue for another 75ms before moving to the next point and repeating the process.

To set up the proper pulse train on the Pockels output line:

- The 'Pulse Potential' would be set to the appropriate value (based upon the 'Ext. Sensitivity' setting) to get a 5 volt output.
- Set the 'Duration' to 25 (ms).
- Set the 'Inter-Pulse Delay' to 75.1ms (75ms of additional data acquisition time before moving to the next location plus 0.1 ms for the time to move the pointing device.)

- Set the 'Number of Pulses' to 5 (one pulse at the start of each new marked point).
- Finally, the 'First Pulse Delay' should be set to 0.

When the 'Update' button is pressed, the composite graph should show the spikes that indicate when the system is moving to the next marked point, and immediately after each spike there should be a pulse of 25ms duration indicating that the Pockels output is being executed.

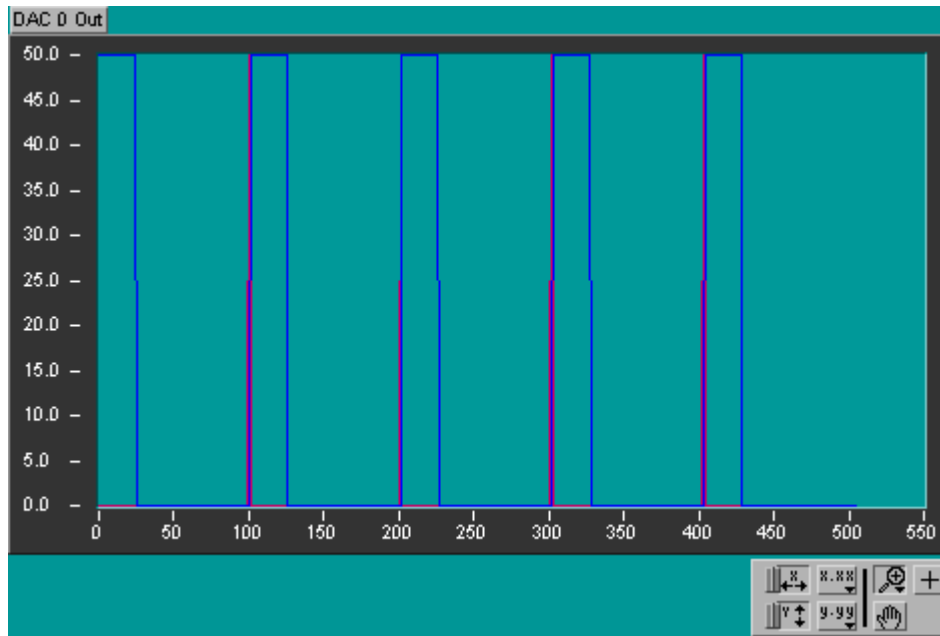


Figure 8.16: DAC Out Composite graph.

8.3 ADC Input Controls

Section 8.2 outlines the process of setting up the various analog output signals and some of the parameters that affect the collection or acquisition of analog signals. This section will cover the remaining controls and dialogs that are used for configuration of **the analog inputs**.

8.3.1 Selecting Input Channels



Figure 8.17: ADC inputs.

Across the top center of the main window of *TriggerSync* are a series of eight buttons with small color boxes just to the right and below these buttons. These buttons are used for specifying which input electrical signals to record with

TriggerSync. To perform a *TriggerSync* acquisition at least one of these buttons must be pressed. When none of the buttons are pressed (selected), the 'Acquire' button will be desensitized (made inactive).

The eight buttons correspond in order from left to right to the first eight inputs on the BNC-2090 box labeled ACH0, ACH1, etc. from left to right.

By left-clicking the mouse on a color box next to one of the input buttons, a color selection dialog will appear that will allow you to select the color associated with that input signal. The selected color will be the color of the button when it is depressed (selected) as well as the color of the graph data for that input.

8.3.2 ADC Configuration

The ADC Configuration dialog contains a variety of parameters that improve the *TriggerSync* dialog readability and control the scaling of the acquired data.

To open the ADC Configuration dialog click on the menu option 'Configuration' and then click on the option 'ADC Configuration...'.

This dialog allows the operator to control several parameters for each of the inputs. There are eight columns of parameters, one column for each of the eight inputs that are available.

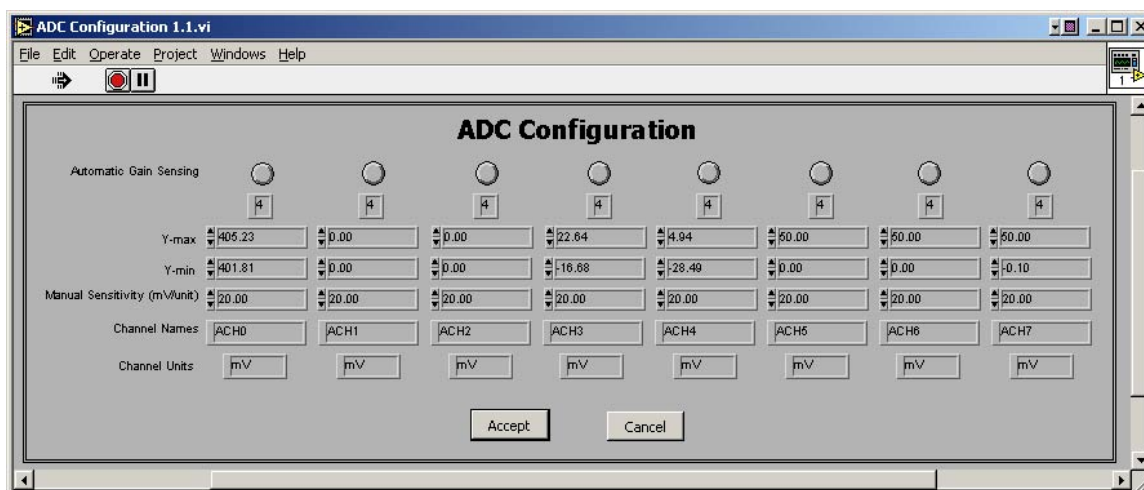


Figure 8.18: ADC Configuration.

The first three parameters 'Automatic Gain Sensing', 'Y-max', and 'Y-min' are only used after data has been collected (an acquisition has been performed) within *TriggerSync* and the program is in the 'Analyze Acquired Data' mode.

The first parameter is labeled '**Automatic Gain Sensing**'. By pressing this button and exiting the dialog, the minimum and maximum data values for the

selected input will be determined from the data for that channel and that minimum and maximum value will be used as the minimum and maximum values for the vertical (y) axis of the graph. In essence, the 'Automatic Gain Sensing' button means to automatically scale the graph to the data. As data is acquired, the graph is automatically scaled to the data, which is why this button is only relevant after the data has been acquired and the program is in the 'Analyze Acquired Data' mode.

To set the vertical (y) axis for a given input channel, enter the desired values into the 'Y-max' and 'Y-min' fields for the selected channels. After exiting the dialog, the data will be redrawn on the graph with the data for the selected input channel scaled based upon the specified 'Y-max' and 'Y-min' values.

Newly acquired data is automatically scaled by default so that the operator will always be able to see the data as it is being acquired (since it won't be impacted by the 'Y-max' or 'Y-min' values). After the data is acquired however and *TriggerSync* is in the 'Analyze Acquired Data' mode, the 'Y-max' and 'Y-min' values can then be set for the desired input channels to the same values and differences in data recorded on those input channels can be more easily compared.

The next field down is labeled as 'Manual Sensitivity (mV/unit)'. Just as the 'Ext. Sensitivity' value for the output protocols was a scale factor for the output voltages, the 'Manual Sensitivity' value is a scale factor applied to the input voltages. Also, just as there is a separate 'Ext. Sensitivity' value for each output channel, there is a separate 'Manual Sensitivity' value for each of the input channels. Mathematically, the data is scaled using the following equation:

displayed value = (input value * 1000)/Manual Sensitivity.

For example, if the input voltage is 0.5 volts, and the 'Manual Sensitivity' is set to 20, then value displayed on the graph will be:

$$25.0 = ((0.5 * 1000) / 20).$$

The second from the bottom field is labeled 'Channel Names'. The string that is entered here will be displayed over the corresponding input button on the main *TriggerSync* window. For example, the third input (ACH2) might be connected to a patch amplifier, so the operator might enter a label such as 'amp' in the 'Channel Names' field for the third input.

The bottom most field is labeled 'Channel Units'. The string that is entered here will be displayed to the right of the color box for the corresponding input button on the main *TriggerSync* window. For example, the third input (ACH2) might be

connected to a patch amplifier, so the operator might enter a label such as 'mV' in the 'Channel Units' field for the third input.

8.4 Point Calibration

8.4.1 Overview

In system configurations where the image scanning galvanometers are not used as the pointing device (for uncaging and so forth), then it is necessary to calibrate the pointing device to the image.

The result of the point calibration process is a map that relates voltage values (for galvanometer based pointing devices) or motor positions (for motor driven pointing devices) to pixel locations on the image. This map is then used within the Mark Points dialog for a fast method by which the operator can specify a location on the image that he/she wishes the pointing device to be located.

It is important to note that since the calibration is dependent upon the pixel locations in the image, any changes to the image generation parameters (pan, zoom, rotation, image resolution, changing objective lenses, and etc.) will necessitate a separate calibration.

At any time during the setup of the calibration procedure, the operator may press the 'Done' button at the bottom of the dialog to exit this dialog.

8.4.2 Calibration Process

To begin the point calibration process, click on the 'Points' menu option in the *TriggerSync* main window and then click on the 'Calibrate Points' option.

If the Mark Points dialog is open when the Point Calibration dialog is opened, the Mark Points dialog will automatically be closed.

The Point Calibration dialog is used in the calibration of various pointing devices to various imaging devices. For example, the operator could calibrate a galvanometer driven pointing device to a galvanometer driven imaging device, or operator could calibrate a galvanometer driven pointing device to a digital camera, and so on.

This discussion will center on the calibration of a galvanometer driven pointing device to a galvanometer driven imaging device.

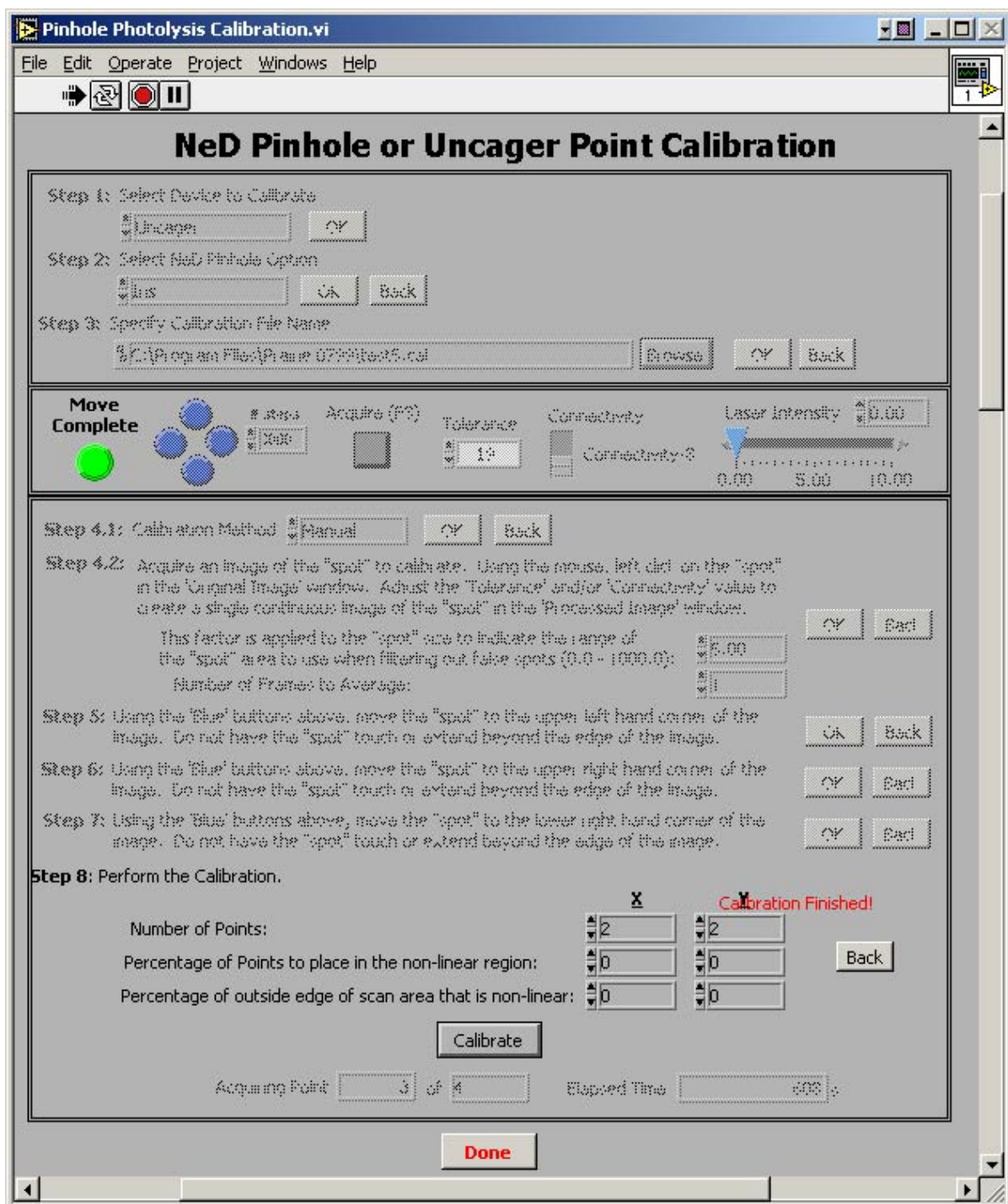


Figure 8.19: Point Calibration dialog

The calibration process is set out in a number of steps that the operator must proceed through in a specific order.

Due to various optical considerations, it may not be possible to see the 'spot' at the extremes of the image field (left, right, top, or bottom). Because of this, the area that will be calibrated is often not the entire visible image area.

Step 1: Device Selection

The first step in the calibration procedure is to select the device for which the calibration will be performed. For most systems, the only available, and hence default option, will be 'Uncager'. This is the generic name given to any pointing device (regardless if it is galvanometer driven or motor driven) that is not the 'NeD Pinhole' device.

- Using the 'Select Device to Calibrate' control, select the desired pointing device.
- Press the 'OK' button to proceed to the next step.

Step 2: NeD Pinhole Option

If the specified device in step 1 is 'Uncager', then this step will be automatically skipped and the procedure will jump straight to step 3.

If however the 'NeD Pinhole' device was selected in step 1, then the operator must specify which NeD Pinhole location to perform the point calibration for. The 'NeD Pinhole' device consists of three locations that may be selected from; Iris, Pinhole, and Confocal Pinhole.

The operator can now either press the 'OK' button to proceed to the next step, or press the 'Back' button to return to the previous step.

Step 3: Calibration File Name

In this step the operator must specify the directory and file name to use for saving the calibration information.

To select the directory and file name:

- Click on the 'Browse' button and then use the pop-up dialog to select the directory and enter the file name to use.
- After the 'Save' button in the pop-up dialog is pressed, the selected directory path and file name will be shown to the left of the 'Browse' button.

- The operator can now either press the 'OK' button to proceed to the next step, or press the 'Back' button to return to the previous step.

Hints on Naming the Calibration File: Since the calibration data is dependent upon many system parameters, it is useful if the calibration file name incorporated enough information so that the operator can keep the different calibration files separate and easily select the proper file.

For example, if the operator is using a 60x water (H₂O) lens at an image resolution of 512x512, and a zoom of 1, he/she might select a calibration file name of 'Lens60-H2O-512x512-zoom1.cal' (the .cal extension will be automatically appended to the operator specified name). Now, if the operator wishes to calibrate the same lens at the same image resolution but at a zoom of 2, he/she might specify a file name of 'Lens60-H2O-512x512-zoom2.cal'.

Step 4.1: Calibration Method

This step allows the operator to specify whether a 'Manual' (Default) or 'Automatic' calibration should be performed. **In general, it is recommended that the operator select the 'Manual' option.**

The operator can now either press the 'OK' button to proceed to the next step, or press the 'Back' button to return to the previous step.

Step 4.2: Setup for 'Automatic' Calibration

If in calibration **Step 4.1** the operator had selected the 'Manual' calibration option, this step would have been skipped.

In its current implementation, for the 'Automatic' point calibration to proceed successfully, the 'spot' image needs to maintain a relatively constant intensity and shape throughout the area of the image to be calibrated. If during the calibration process the software can not successfully determine the location of the 'spot' in the image, it will pop up a dialog that will allow the operator to select the 'spot' image location manually. The software will then continue and attempt to find subsequent 'spot' image locations automatically.

For the 'Automatic' point calibration procedure to work, it is necessary for the operator to "tell" the program where the 'spot' image is in the field of view and then to set several controls that help the program to characterize the 'spot' image.

- **First**, acquire an image of the 'spot' by pressing the 'Acquire' button located below step 3. Depending upon the system configuration, it may be necessary to open a hard shutter in front of the 'spot' laser. In the main

window of *TriggerSync* there is a 'Shutter (F1)' control in the lower left hand corner that may be used to open/close the hard shutter in front of the 'spot' laser. If the system configuration includes a Pockels controller in front of the 'spot' laser, there is a 'Laser Intensity' control in the area below step 3. Use this control to increase the laser intensity until the 'spot' can be seen in the image.

If it is necessary to physically move the 'spot' location use the four blue buttons located to the left of the 'Acquire' button.

The four blue buttons are oriented in a diamond pattern to indicate the direction the pointing device should move when the button is pressed. For example, if the top most blue button is pressed, the pointing device should move up or towards the top of the current image by the amount specified in the '# steps' field.

The selected pointing device will move an amount specified by '# steps'. The value to use for '# steps' is dependent upon the type of pointing device being used. For a galvanometer based pointing device, the '# steps' field should be specified in millivolts. So a value of 200 in this field indicates a desired movement equal to 200 mV from the current location. For a motor driven pointing device, the '# steps' field should be specified in motor steps. So a value of 55 in this field indicates a desired movement equal to 55 motor steps from the current location.

When the 'Acquire' button is pressed, two image windows will appear. One is labeled 'Original Image' and the other is labeled 'Processed Image'. Within the 'Original Image' window position the mouse over the location of the 'spot' and click the left mouse button. In the 'Processed Image' window you should see a binary representation of the 'spot'. A binary image is an image where a pixel only has two values, either black or white. So the 'Processed Image' window should show a primarily black image with a small white area that represents the 'spot'.

- **Second**, adjust the values for the 'Tolerance' and 'Connectivity' controls until the 'Processed Image' shows a valid representation of the 'spot' from the 'Original Image' window. This process is strictly empirical; there are not pre-determined values that will automatically generate a good 'Processed Image'.
- **Third**, specify the 'Number of Frames to Average' (the options are 1, 2, 4, or 8). If the image is noisy, sometimes averaging 2 or more frames together will result in a cleaner image.

- **Fourth**, define a size factor to be used during the calibration process. During this setup part of the 'Automatic' calibration process, the size of the 'spot' is determined. Then, during the actual calibration, the size is used as a filter on the image if more than one 'spot' is found. This size factor allows the operator to specify a value that will be applied to the measured spot size as a plus/minus value to produce a range of sizes that would constitute the correct 'spot'. For example, if the 'spot' in the 'Processed Image' window is very small, on the order of a few pixels, then a small value of say 5 should be set in this field. If however the 'spot' is large, on the order of hundreds of pixels, then this field should be set to a larger value such as 50.
- Either press the 'OK' button to proceed to the next step, or press the 'Back' button to return to the previous step.

Step 5: Define Upper Left Hand Corner

As soon as this step is reached, an image window will be displayed with the label 'Upper Left Hand Corner Image'. In this step, the operator must manually position the 'spot' in the ***upper left hand corner*** of the area to calibrate.

- Manually move the 'spot' location using the four blue buttons located to the left of the 'Acquire' button.

The four blue buttons are oriented in a diamond pattern to indicate the direction the pointing device should move when the button is pressed. For example, if the top most blue button is pressed, the pointing device should move up or towards the top of the current image by the amount specified in the '# steps' field.

The selected pointing device will move an amount specified by '# steps'. The value to use for '# steps' is dependent upon the type of pointing device being used. For a galvanometer based pointing device, the '# steps' field should be specified in millivolts (mV). So a value of 200 in this field indicates a desired movement equal to 200 mV from the current location. For a motor driven pointing device, the '# steps' field should be specified in motor steps. So a value of 55 in this field indicates a desired movement equal to 55 motor steps from the current location.

The process is to move the 'spot' left and/or up using the 'Blue' buttons and then to use the 'Acquire' button to capture a new 'spot' image.

- Once the 'spot' is in the desired location (the Upper Left Corner), press the 'OK' button to proceed to the next step or press the 'Back' button to return to the previous step.

Step 6: Define Upper Right Hand Corner

When this step is reached, an image window will be displayed with the label 'Upper Right Hand Corner Image'. In this step, the operator must manually position the 'spot' in the ***upper right hand corner*** of the area to calibrate.

- Manually move the 'spot' location, using the four blue buttons located to the left of the 'Acquire' button.

The process is to move the 'spot' left or right using the 'Blue' buttons and then to use the 'Acquire' button to capture a new 'spot' image. You will notice that during this step the 'Blue' buttons that control movement of the 'spot' up and down have been desensitized. This forces the operator to only make left or right movement corrections when defining the upper right hand corner location relative to the upper left hand corner location.

- Once the 'spot' is in the desired location, press the 'OK' button to proceed to the next step or press the 'Back' button to return to the previous step.

Step 7: Define Lower Right Hand Corner

When this step is reached, an image window will be displayed with the label 'Lower Right Hand Corner Image'. In this step, the operator must manually position the 'spot' in the ***lower right hand corner*** of the area to calibrate.

- Manually move the 'spot' location, using the four blue buttons located to the left of the 'Acquire' button.

The process is to move the 'spot' left or right using the 'Blue' buttons and then to use the 'Acquire' button to capture a new 'spot' image. You will notice that during this step the 'Blue' buttons that control movement of the 'spot' left and right have been desensitized. This forces the operator to only make up or down movement corrections when defining the lower right hand corner location relative to the upper right hand corner location.

- Once the 'spot' is in the desired location, press the 'OK' button to proceed to the next step or press the 'Back' button to return to the previous step.

Step 8: Perform the Calibration

Before performing the actual calibration, the operator must specify a few more control values.

- The operator must specify the 'Number of Points' in the x and y dimension to calibrate. Depending upon the desired accuracy of the pointing device calibration, typically a value of 2 in each of these fields will yield acceptable results. At a value of 2 points in x and y, the calibration is essentially being performed at the four corners that define the calibration area.

The four remaining controls; 'Percentage of Points to place in the non-linear region' (x and y) and 'Percentage of outside edge of scan area that is non-linear' (x and y) are only used when the imaging device has non-linear movement.

Note: *The standard imaging galvanometers that are used exhibit linear motion in the region that Prairie uses them so typically all four of these fields would be set to 0.*

If non-linear behavior is found in either the x and/or y axes of the imaging galvanometers, these fields could be used to provide for more accurate calibration of the pointing device.

Note: *If non-linear behavior is found, it would most likely be in the x axis (the fast scan axis).*

The control 'Percentage of Points to place in the non-linear region' is used to indicate what percentage of the total number of points in the x or y dimension to allocate to the non-linear region. For example, if non-linear movement is in the x axis and the operator had indicated that 20 points should be used in the 'Number of Points' control for the x dimension, and 40 is entered into the control 'Percentage of Points to place in the non-linear region' for the x dimension, then of the 20 points, 8 points (40% of 20) will be in the non-linear region on both the left and right side. So of the 20 total points, 16 points will be in the non-linear region and 4 points will be in the linear (center) section. This will result in a higher density calibration in the non-linear region and should result in higher calibration accuracy of the pointing device in that region.

The control 'Percentage of outside edge of scan area that is non-linear' is used to indicate the percentage of the total defined scan area in the x or y dimension that corresponds to the non-linear region. For example, if non-linear movement is in the x axis and the image is 512 pixels in the x axis and it is noticed that the non-linear region extends 50 pixels in on either side, then the value for the control 'Percentage of outside edge of scan area that is non-linear' would be 10 (50 is approximately 10% of 512).

- After these values have been set, press the '**Calibrate**' button to begin the calibration.

As the calibration proceeds, there are a number of fields directly below the 'Calibrate' button that indicate the status of the calibration. The first two fields indicate the current point number being calibrated relative to the total number that need to be calibrated. The third field indicates the elapsed time since the 'Calibrate' button was pressed.

8.4.2.1 Manual Calibration

If the 'Manual' calibration method had been selected in Step 4.1, then starting at the first calibration point, a new dialog will appear that shows the image of the 'spot' and just two buttons. A field below the image window in this dialog indicates which calibration point is currently displayed (e.g. 1 of 4).

- Place the red cursor in the image window over the center of the 'spot' in the image window. Use the tools on the lower left hand of the image window to zoom the image window to provide for greater accuracy in placement of the red cursor.
- Once the cursor is placed, press the '**Accept**' button. After pressing the '**Accept**' button, the software will move the pointing device to the next location, acquire a new image, and then display it in this same window and wait for the operator to place the red cursor over the 'spot' and once again press the 'Accept' button. This process will repeat for all of the points in the calibration setup or until the '**Abort Calibration**' button is pressed.

Note: If the operator presses the '**Abort Calibration**' button, then this dialog will be dismissed and the calibration procedure will terminate.

8.4.2.2 Automatic Calibration

If the 'Automatic' method had been selected in Step 4.2, then starting with the first calibration location the software will move the pointing device to the desired location, acquire the 'spot' image, and then attempt to automatically find the 'spot' in the image. If the 'spot' is found, then the application will automatically proceed to the next location and repeat the steps. This will continue until all of the calibration locations have been reached.

The Automatic Calibration will pause in its operation if it is unable to find the spot or if it finds more than one spot in the image. If this occurs, a new dialog exactly like the one described in the previous section on manual calibration appears. This allows for the opportunity to manually indicate where the spot is located. After placing the red cursor over the 'spot' location, if the operator presses the '**Accept**' button, the dialog will disappear and the software will continue attempting to automatically perform the calibration. At any location where the

software runs into a problem in finding the spot, this manual override dialog will be displayed allowing for a means of continuing with the calibration.

If the **'Abort Calibration'** button is pressed, then this dialog will be dismissed and the calibration procedure will terminate.

After the calibration has completed, press the **'Done'** button to exit this dialog.

8.5 Marking Points

In the main window of *TriggerSync*, select the **'Points'** menu option and then select the 'Mark Points' option, this will bring up the 'Mark Points' dialog. The 'Mark Points' dialog is used to create a list of locations for performing uncaging experiments.

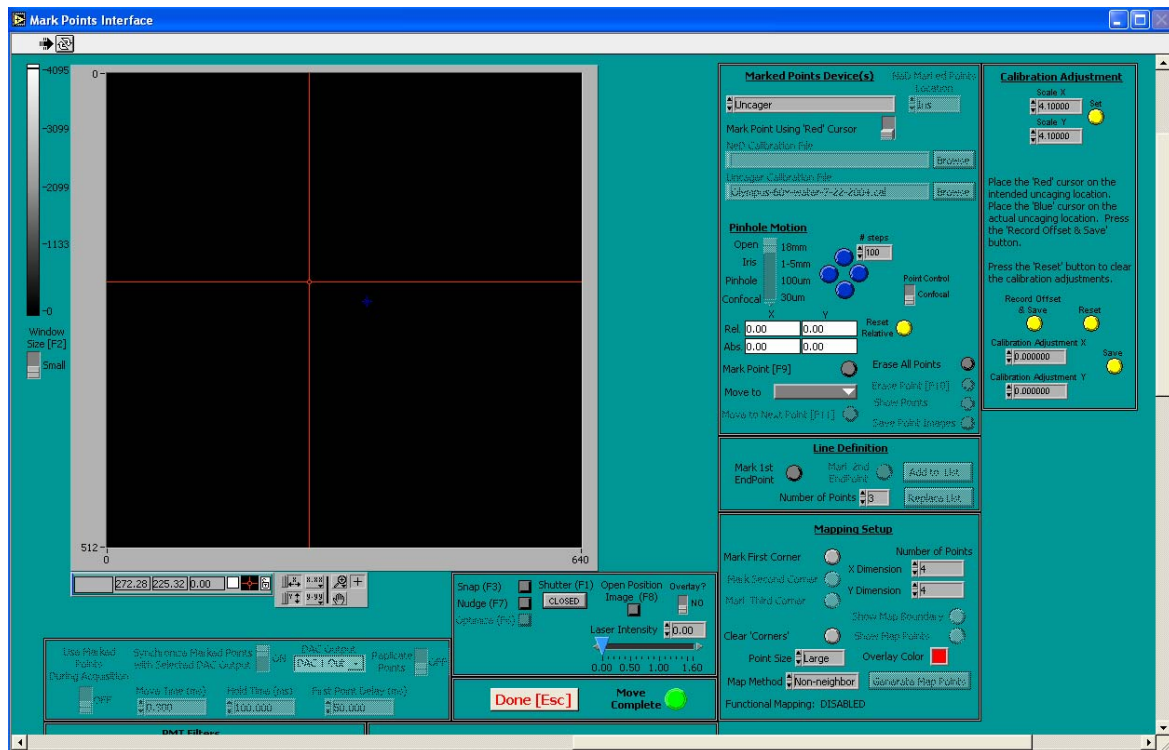


Figure 8.20: Mark Points window.

The image window occupies the largest part of this dialog. There are two cursors displayed in the image window, one red and one blue. When two pointing devices are present on a system, a third (yellow) cursor may also be present dependent upon the option selected in the 'Marked Points Device(s)' control. Since the blue cursor is used to indicate the current position of the selected pointing device, it should not be moved. Using this dialog, you will notice the

placement of the blue cursor changing automatically in response to changes in the physical position of the selected pointing device.

The red cursor is intended to be the primary method of selecting locations with the various marked points generation tools.

8.5.1 Calibration Files and General Controls

The section of the dialog along the upper right hand side contains the controls for selecting the calibration file for the selecting 'pointing' device. The 'Marked Point Device(s)' control is used for selecting the desired 'pointing' device. Only valid options will be available for selection. For the Ultima system, the only available pointing device option is the 'Uncager' option. For an Ultima that contains only one set of galvanometers, it is possible for a separate Prairie Photolysis Head to be installed. This Photolysis Head would also be selected as the 'Uncager'.

To the right of the 'Marked Points Device(s)' control is the control 'NeD Marked Points Location'. This control has three options:

- A) Iris
- B) Pinhole
- C) Confocal

The selection will determine which NeD Pinhole option will be used for generating the marked points.

The switch directly below the 'Marked Points Device(s)' control is 'Mark Point using 'Red/Blue' Cursor'. Typically, the operator will leave this control set to the '**Red**' cursor option. In this mode, the red cursor within the image window is used with the various marked points generation tools. When the 'Blue' cursor option is selected the blue cursor within the image window is used with the various marked points generation tools. When the 'Blue' cursor option is selected, the selected pointing device must have its physical position changed (for example using the four blue buttons next to the 'Pinhole Motion' control) to indicate the position to use in conjunction with the various marked points generation tools.

The 'NeD Calibration File' field is used to indicate the calibration file to use for computing the marked point positions when the NeD Pinhole device is being used with a confocal image. When the NeD Pinhole is being used with a digital camera image, the necessary calibration numbers are stored in a configuration file (ControlNeD.ini). To select a calibration file, click on the 'Browse' button next to the 'NeD Calibration File' field and select the desired file.

The 'Uncager Calibration File' field is used to indicate the calibration file to use for computing the marked point positions when the Uncager device is being used with a confocal image or with a digital camera image. This calibration file is one of the files generated in the Point Calibration dialog. To select a calibration file, click on the 'Browse' button next to the 'NeD Calibration File' field and select the desired file.

The 'Pinhole Motion' control will only be active on older Prairie systems that include a NeD Pinhole (for near field detection).

To the right of the 'Pinhole Motion' control is a set of four blue buttons and a control labeled '# steps'. These blue buttons are used to physically move the selected pointing device. The selected pointing device will move an amount specified by '# steps'.

The value to use for '# steps' is dependent upon the type of pointing device being used. For a galvanometer-based pointing device, the '# steps' field should be specified in millivolts (mV). So a value of 200 in this field indicates a desired movement equal to 200 mV from the current location. For a motor driven pointing device, the '# steps' field should be specified in motor steps. So a value of 55 in this field indicates a desired movement equal to 55 motor steps from the current location.

The four blue buttons are oriented in a diamond pattern to indicate the direction the pointing device should move when the button is pressed. For example, if the top most blue button is pressed, the pointing device should move up or towards the top of the current image by the amount specified in the '# steps' field.

Based upon the accuracy of the pointing device calibration, it will most likely not be necessary for the operator to use the blue buttons to position the pointing device manually. Typically, just the 'red' cursor will be used.

Below the four blue buttons are four fields that indicate the location of the current pointing device. The bottom most two fields indicate the absolute position of the pointing device in the x and y dimension. The number that is displayed within these fields is dependent upon the pointing device. The top two fields indicate the relative position of the pointing device with regard to the absolute position.

For a galvanometer based pointing device, the values displayed in these fields are in volts. For a motor driven pointing device, the values displayed in these fields are in motor steps.

Whenever the position of the selected pointing device is modified using the previously described blue buttons, you will see the blue cursor in the image window move, and the values displayed in the relative position and absolute

position fields will change as well. In addition, any action that results in a new physical position for the selected pointing device will cause the blue cursor in the image window to move (to indicate where the pointing device is currently located), as well as cause the values displayed in the relative position and absolute position fields to be updated.

To the right of the relative position fields is a yellow button, 'Reset Relative'. When this button is pressed, the values in the relative position fields will be reset to 0.

8.5.2 Marking points – Basic Controls

Immediately below the pointing device location information are the basic controls for marking points, moving between marked points, deleting marked points, displaying marked points, and saving marked points information.

The button labeled 'Mark Point (F9)' is the basic control used for marking a location on the image for further study using the selected pointing device. The (F9) in parenthesis indicates that there is a hotkey on the keyboard linked to this control. So the operator may press the F9 key and it will result in the same action as if the 'Mark Point (F9)' button itself had been pressed.

The actual location that will be marked when the 'Mark Point' button is pressed is dependent upon the setting of the 'Mark Point Using 'Red/Blue' Cursor' control previously described. If this control is set to the 'Red' cursor option, then the operator merely places the red cursor in the image window at the desired location to mark and presses the 'Mark Point' button. If this control is set to the 'Blue' cursor option, then the operator must physically move the location of the pointing device using the blue buttons or the 'Nudge (F7)' control (discussed later) to the desired location and then press the 'Mark Point' button. As you can see, using the 'Blue' cursor option is much slower which is why it is recommended to use the 'Red' cursor option.

The list of marked points is maintained even after the *TriggerSync* program has quit running. The next time *TriggerSync* is executed, the last list of marked points will still be present in the program.

As locations are 'marked', there is a control immediately below the 'Mark Point' button that contains a list of the current marked points. Immediately to the left of the control is the string 'Move to'. As more locations are marked, this control will automatically update. The operator may use this control to immediately move the selected pointing device to any of the locations that have been previously marked. To do this, simply click on the control and select the desired point number. As the pointing device is moved you will notice the blue cursor in the image window move to the point location just selected. Also, the values for the

absolute position will be updated and the values for the relative position will be zeroed.

Below the 'Move To' control is a button labeled 'Move to Next Point (F11)'. Just as the 'Mark Point' control had F9 as a hotkey for its action, this control uses F11 as a hotkey. By pressing this button (or F11), the software will automatically select the next point in the marked points list from the current value. For example, if 'Point 4' is the current value for the 'Move to' control and the operator presses the 'Move to Next Point' button, the 'Move to' control will increment to 'Point 5' and the blue cursor in the image window will move to the new point location just selected. Also, the values for the absolute position will be updated and the values for the relative position will be zeroed. When the end of the marked point list is reached, it will automatically wrap around and start over with Point 0.

To the right of the controls to mark points is the 'Erase All Points' button. When this button is pressed, all previously marked points are deleted.

Other actions besides pressing the 'Erase All Points' button will also result in all marked points being deleted. If the 'NeD Calibration File' or the 'Uncager Calibration File' is changed, any previously marked points will be deleted. In addition, within the 'Mapping Setup' section of the Mark Points dialog, when the 'Generate Map Points' button is pressed any previous marked points will be deleted (this will be discussed in more detail in that section). Also, within the 'Line Definition' section of the Mark Points dialog, when the 'Replace List' button is pressed any previous marked points will be deleted (this will be discussed in more detail in that section).

Below the 'Erase All Points' button is the 'Erase Point (F10)' button. This control is used to delete a single marked point. When this button is pressed, a dialog will appear asking the operator to select the marked point to delete. The operator merely selects the desired point and presses the 'OK' button. If the operator does not wish to delete a point, he/she may press the 'Cancel' button. If the 'OK' button has been pressed, then the operator will be prompted again to make certain that they wish to delete this point. If 'YES' is pressed, the point is deleted. If 'NO' is pressed, the point is not deleted.

Below the 'Erase Point' button is the 'Show Points' button. When this button is pressed it will display a new image window with the current marked points locations indicated on the image in the color specified in the 'Overlay Color' control near the bottom right hand corner of the Mark Points dialog.

Below the 'Show Points' button is the 'Save Point Images' button. When this button is pressed, it will display a dialog that presents the operator with four check boxes:

- A) Save Open Image
- B) Save Open Image with All Points Marked
- C) Save Individual Open Image with Each Point Marked
- D) Save Cursor Locations

The operator may select any combination of these options. After selecting the desired options, press the 'Cancel' button to exit the dialog or press the 'OK' button to save the desired data.

If the 'OK' button is pressed, the operator will be prompted to select a directory and base filename to use for saving the desired data.

If **Option A** is selected, then the current image will be saved with the specified file name.

If **Option B** is selected, then the current image will be saved and superimposed on the image will be indicators for all of the marked points. This file will use the specified file name and have '-AllPoints' appended to the name.

If **Option C** is selected, then a separate file will be saved with the current image and each of the marked points individually superimposed on the image. These files will use the specified file name and have '-Pointn' appended to the name, where 'n' would go from 0 to the number of marked points.

If **Option D** is selected, then a text file is created that contains two columns of numbers. The first column is the x cursor position and the second column is the y cursor position for each marked point. This file uses the specified file name with a '.dat' extension.

8.5.3 Line Definition

This section of the Mark Points dialog contains controls to help the operator create a straight line of marked points. Currently, this option only works with the 'Mark Point Using 'Red/Blue' Cursor' control set to the 'Red' option.

To create a line of marked points, first place the red cursor in the image window where you wish to start the line. Then press the 'Mark 1st EndPoint' button. Next, place the red cursor in the image window where you wish to end the line. Then press the 'Mark 2nd EndPoint' button. Below the 'Mark 2nd EndPoint' button is a control labeled 'Number of Points'. Use this control to specify the number of points you wish to create on this line including the two endpoints. The minimum number of points on a line is 3.

After selecting the desired number of points, the operator may either press the 'Add to List' button or the 'Replace List' button. If the 'Add to List' button is pressed, the new marked points that form the defined line will be added to the current list of marked points. This allows the operator to string together a series of marked points that might consist of randomly specified locations along with one or more straight line segments and so on. If the 'Replace List' button is pressed, then any previously defined marked points will be deleted and the new list of marked points will only consist of those from the line definition.

8.5.4 Mapping Setup

This section of the 'Mark Points' dialog contains controls to help the operator create a grid of marked points. Currently, this option only works with the 'Mark Point Using 'Red/Blue' Cursor' control set to the 'Red' option.

While this grid of marked points may be used in any experimental way that any of the other marked points may be used, within the *TriggerSync* program a grid of marked points provides a new data acquisition and analysis option. This new option is referred to as **Functional Mapping**.

To create a grid of marked points:

- Position the red cursor in the image window to indicate the location of what would be the upper left hand corner of the grid.
- Press the **'Mark First Corner'** button.
- Position the red cursor in the image window to indicate the location of what would be the upper right hand corner of the grid.
- Press the **'Mark Second Corner'** button. If this second corner location is not to the right of the first corner, then a message explaining this problem will be displayed and the operator must start the grid definition process over.
- Position the red cursor in the image window to indicate the location of what would be the lower right hand corner of the grid.
- Press the **'Mark Third Corner'** button.

Since the grid must be rectangular (right angles at the corners), the actual grid coordinates are determined using the following logic. The defined locations of the first and second corner define one side of the grid. The second side is defined by taking the distance between the second and third corners (this defines the length of the second side) and determining the new location of the third corner that will create a right angle between the first side and the second side. The remaining sides are easily calculated now.

At any time, the operator may press the '**Clear 'Corners'**' button to delete the previously defined corner locations and allow the operator to redefine the grid limits.

The defined grid of marked points can be either oriented along the image axes or at a random angle relative to the image axes.

To define a grid that is oriented along the image axes:

- Place the red cursor in the image window at the location of the desired upper left hand corner
- Press the '**Mark First Corner**' button.
- Next, only move the vertical line of the red cursor to the location of the second corner (this will guarantee that this line is oriented with the image x axis)
- Press the '**Mark Second Corner**' button.
- Lastly, only move the horizontal line of the red cursor to the location of the third corner (this will guarantee that this line is oriented with the image y axis)
- Press the '**Mark Third Corner**' button.

Once the three 'corners' of the grid have been defined, the '**Show Map Boundary**' button becomes active. If the operator presses this button, a separate image window will appear with a box drawn over the section of the image showing the grid boundaries. The color of the box is determined by the color specified in the 'Overlay Color' control just below the 'Show Map Boundary' button. This allows the operator to visualize where exactly the grid will be created.

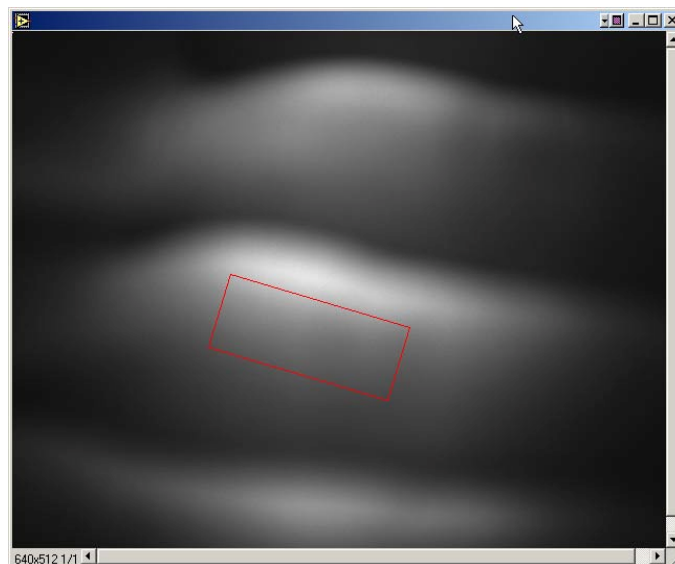


Figure 8.21: Show map boundary.

Next, specify the number of marked points to create in the x and y dimension of the grid. These values are set using the 'X Dimension' and 'Y Dimension' controls. Currently, the number of marked points in the x dimension must be a multiple of 4 (4,8,12,16,...) and the number of marked points in the y dimension must be a multiple of 2 (2,4,6,8,...).

The spacing between successive points will be dependent upon the size of the specified grid and the number of points specified in the x and y dimension.

After the number of points in the x and y dimension have been specified, the operator needs to select the 'Map Method'. Currently, three options are available:

Option A (Sequential): The order that the marked points will be generated for the grid will be a simple progression starting at the upper left hand corner location and increasing across to the upper right hand corner, then dropping down and continuing from the left side to the right side and so on.

Option B (Non-neighbor): The order that the marked points will be generated for the grid will follow a mathematical progression that is designed to keep successive points from being too close to each other. The following section defines the method used for computing these locations.

To eliminate the effects of desensitization of receptors by previous uncaging at nearby pixels it is necessary to pseudo-randomize the uncaging positions. If the area that you wish to perform the uncaging in is composed of $2N_x \times 2N_y$ pixels, the position of the scanner ($X(i)$, $Y(i)$) at the i th uncaging ($i = 0, 1, \dots, 4 \times N_x \times N_y$) is determined as

$$X(i) = N_x * \text{mod}[i, 2] + P_x(\text{int}[\text{mod}[i, 4 \times N_x] / 4])$$

$$Y(i) = N_y * \text{int}[\text{mod}[i, 4] / 2 + \text{int}[i / (4 \times N_x)]]$$

where P_x is, for example, $P_x(i) = (0, 4, 2, 6, 1, 5, 3, 7)$ when $N_x = 8$.

In the statements listed above, $\text{mod}[]$, is the modulus function. This function returns the remainder of the division of the first argument by the second argument. The function, $\text{int}[]$, returns the integer value of its argument. For example $\text{int}[1] = 1$, $\text{int}[1.3] = 1$, $\text{int}[1.5] = 1$, and $\text{int}[1.99] = 1$.

For this algorithm to work, the number of uncaging positions in the x dimension must be evenly divisible by 4 and the number of uncaging positions in the y dimension must be evenly divisible by 2.

The following table shows an example of the **order of uncaging positions** where $N_x = 8$, $N_y = 4$, and $P_x(i) = (0,4,2,6,1,5,3,7)$.

		X Pixel																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Y Pixel	1	1	17	9	24	5	21	13	29	2	18	10	26	6	22	14	30	
	2	33	49	41	57	37	53	45	61	34	50	42	58	38	54	46	62	
	3	65	66	
	4	
	5	3	19	11	27	7	23	15	31	4	20	12	28	8	24	16	32	
	6	35	51	43	59	39	55	47	63	36	52	44	60	40	56	48	64	
	7	67	68
	8

Table 3: "Non-Neighbor" order of uncaging example.

Option C (Random): The order that the marked points will be generated for the grid will be random.

After selecting the **'Map Method'** the operator must press the **'Generate Map Points'** button. It is only when this button is pressed that the actual marked points for the defined grid will be generated.

Once the **'Generate Map Points'** button has been pressed, the **'Show Map Points'** button becomes active. If the operator presses this button, a separate image window will appear with a each of the marked points within the grid area shown. The color of the marked points is determined by the color specified in the **'Overlay Color'** control just below the **'Show Map Boundary'** button. This allows the operator to visualize where exactly the marked points occur.

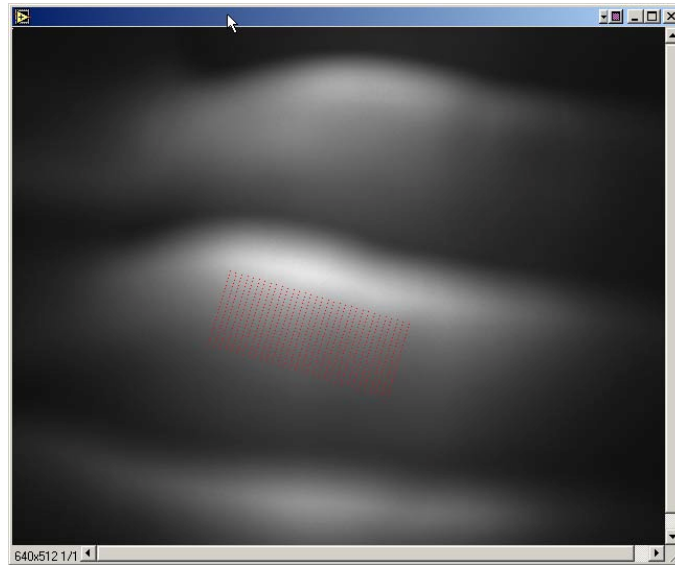


Figure 8.22: Show map points.

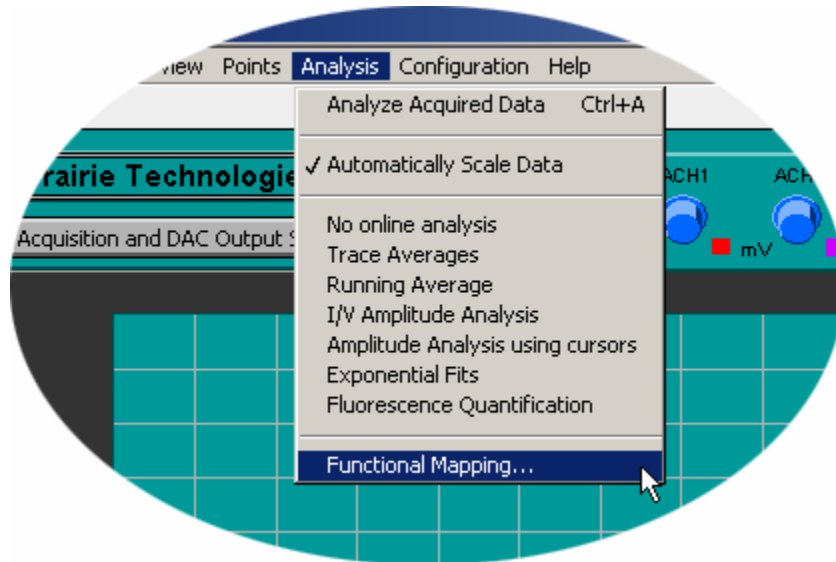
As soon as the **'Generate Map Points'** button is pressed, any previously defined marked points will be deleted. Directly below the 'Map Method' there is a field labeled 'Functional Mapping' that indicates whether the current marked points list will allow a Functional Mapping acquisition (Enabled) or will not allow a Functional Mapping acquisition (Disabled).

If a grid of marked points is to be used with either random marked points and/or a line of marked points, the Functional Mapping acquisition will be turned off. To use a grid of marked points along with either random marked points and/or a line of marked points, first define the grid marked points, and then add the other desired marked point locations.

As soon as one or more marked points are added to the grid definition (or one or more marked points are deleted from those created in the grid definition), the field labeled 'Functional Mapping' will indicate 'Disabled'.

The most recently defined grid corners will be remembered, so that experimenting with different 'Map Methods' can be done by simply making the desired change and pressing the **'Generate Map Points'** button without having to redefine the bounding corners.

8.5.5 Functional Map Display



Click on the **'Functional Mapping'** selection in the **'Analysis'** menu as shown above.

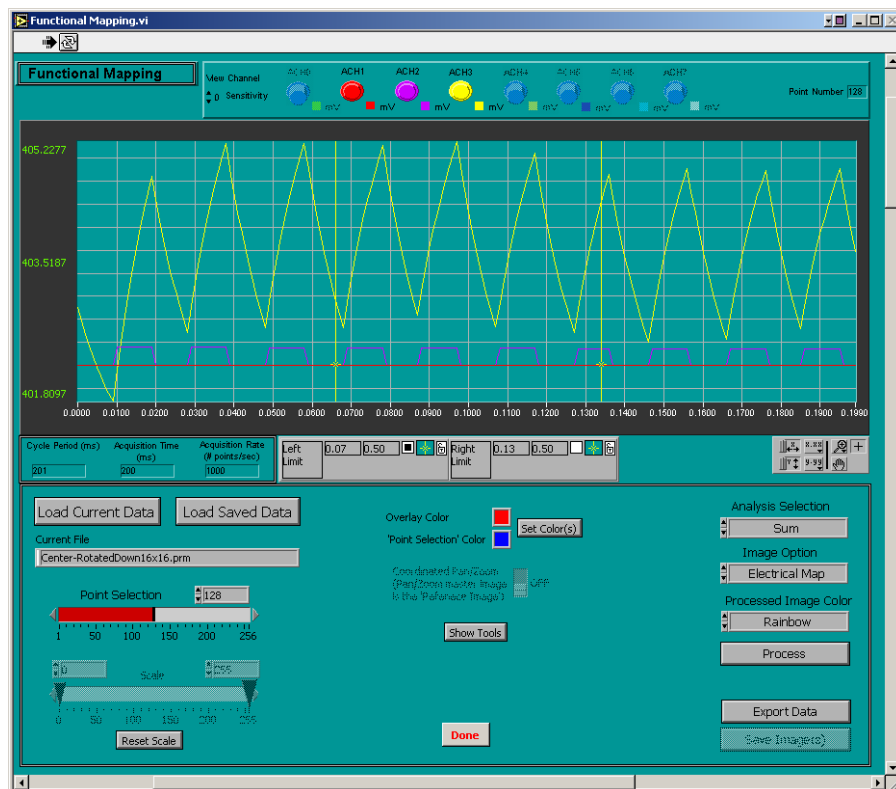
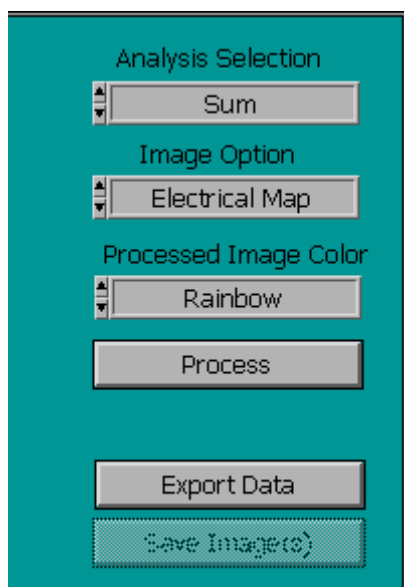


Figure 8.23: Functional Map Display/Analysis.

If a functional mapping experiment has just been conducted the most recently collected data will be displayed. Otherwise use the 'Load Saved Data' button to read in a data file.



- Use the pair of vertical cursors on the electrical data to define the window in time that corresponds to the data you wish to use in your map.
- Select the arithmetic operator under the '**Analysis Selection**'. Choices are average, maximum, minimum, and sum.
- '**Image Option**' offers two choices:
 - 'Electrical Map' - creates a map of the entire rectangular region by means of interpolation to fill in the pixels that were not uncaged directly.
 - 'Pixel Replacement' - replaces the individual pixels that were uncaged only, with no interpolation.
- Select the '**Processed Image Color**' of your choice. This is for display purposes only and has no effect on the data. The color can also be changed after the map has been generated.
- '**Process**' causes the functional map(s) to be generated. The reference image collected with the PMTs will also be displayed for reference along with a calibrated intensity color scale

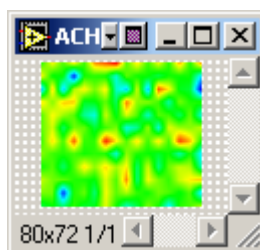


Figure 8.24: Functional Map example.

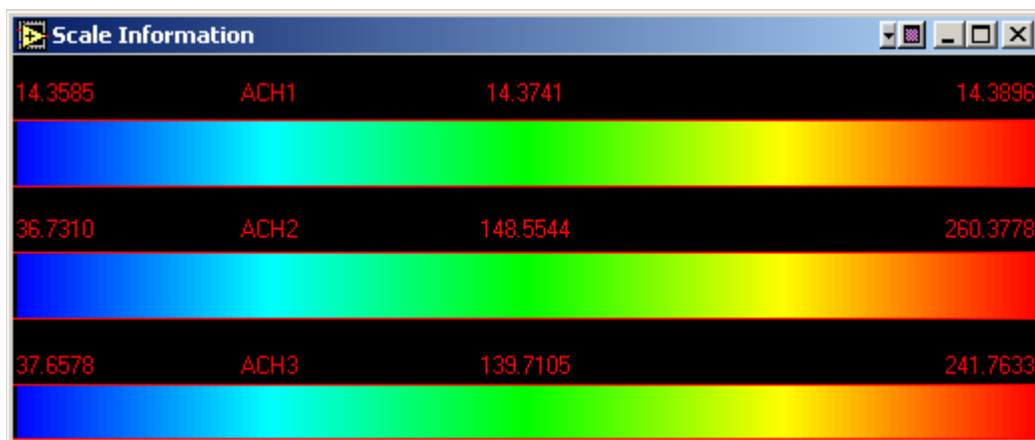


Figure 8.25: Functional Map Calibrated Display scale.

8.5.6 Miscellaneous Controls

Located below the lower right hand corner of the image window is a collection of miscellaneous controls.

- When the **'Snap (F3)' button** is pressed a new image will be acquired. If *TriggerSync* is running in conjunction with a Confocal system, then pressing the 'Snap' button will cause the Confocal software to scan a new image using the scan parameters currently defined scan parameters in the Confocal software. After the image has been acquired, it will be automatically displayed in the image window in the Mark Points dialog.
- The **'Nudge (F7)' button** is used to position the selected pointing device at the location of the red cursor in the image window. This could be useful for validating the accuracy of the calibration of the pointing device.

To use this feature:

- Place the red cursor on the location within the image where the pointing device is to be moved.
- Press the 'Nudge' button (you should see the blue cursor move and the values in the absolute and relative fields updated as well).
- Make the necessary control changes to actually acquire the image of the pointing device (the 'spot' image).
- Acquire an image by pressing the 'Snap' button.

If the calibration is accurate, then the location of the 'spot' in the image field should correspond to the location of the red cursor.

If your system configuration includes a separate Pockels control on the 'uncaging' laser, there will also be slider control in this section labeled **'Laser**

Intensity'. The operator would use this control to adjust the voltage of the uncaging Pockels.

If the selected pointing device is motor driven, the '**Optimize (F6)**' button will be activated (for galvanometer based pointing devices this control is desensitized). This control allows the operator to fine tune the calibration of the motor driven pointing device.

To use this feature:

- 'Nudge' the pointing device to the desired location.
- Acquire an image of the pointing device.
- Move the red cursor from its original position and center it on the 'spot' from the pointing device.
- Press the 'Optimize' button.

This process should eliminate small movement errors that sometimes occur with motor driven pointing devices.

8.5.7 Use Marked Points During Acquisition

This feature allows for very high speed positioning of the pointing device, for this reason this feature is only available if the selected pointing device is driven by galvanometers.

This feature allows the operator to position the pointing device at multiple different locations during a single acquisition cycle. This also means however that the electrical data collection for these multiple locations will be strung together in a single acquisition cycle. This is distinct from other acquisition modes that use the marked points where there is a complete cycle (or more than one) of data acquisition for each marked point.

- If the operator wishes to perform an acquisition where all of the marked points will be visited in a single cycle, then set the control '**Use Marked Points During Acquisition**' to 'On'.
- The control '**Marked Point Hold Time (ms)**' is used to specify how long the pointing device should stay at each marked point during this special acquisition mode.
- The control '**Marked Point Move Time (ms)**' is used to indicate how long it physically takes for the pointing device to move from one marked point to the next. This value defaults to 0.1ms since it takes approximately 100 microseconds for the two galvanometers to move and stabilize between two relatively close locations. If the spacing between the marked points

becomes too large, it may be necessary to increase this value to 0.2 or 0.3ms.

- The control '**Marked Point First Point Delay (ms)**' is used to indicate an additional time delay at the start of the experiment (in addition to the 'Marked Point Hold Time') before moving to the second marked point. As its name implies, this value is only applied to the first marked point.
- The control '**Synchronize Marked Points with Selected DAC Output**' is used to automatically synchronize the movement from one marked point to the next with a pulse/train defined for the selected DAC Output specified by the control 'DAC Output'. Typically, the selected DAC Output will be used to control a Pockels device on the laser controlled by *TriggerSync*. By turning on this option, the software will automatically generate the timing information for the movement between the specified marked points to be properly synchronized with the Pockels control.
- The control '**Replicate Points**' when turned on and the control '**Synchronize Marked Points with Selected DAC Output**' is turned on, will automatically replicate the currently defined mark points to match the number of pulses defined for the DAC Output that is being used for synchronization. For example, let's say that the currently defined pulse train for the selected DAC Output has 12 pulses. Now let's say that there are currently five(5) marked points. If the '**Replicate Points**' control is on, then when the 'Acquire [F5]' button is pressed or the *TriggerSync* acquisition is started, the software will see that only five points have been marked but there are 12 pulses that need to be synchronized with. So the software will automatically (temporarily – just for the acquisition) loop through the five marked points again, which will bring the new total of marked points to 10, which is still two shy of what is needed, so the software will add the first two points again. Now the sequence of points during the acquisition will be (1,2,3,4,5,1,2,3,4,5,1,2).

9 Acquisition Scenarios

One of the most powerful aspects of the ULTIMA system is the ability to collect fluorescence data in synchrony with Uncaging and/or electrophysiology recording. Running *TriggerSync* and *PrairieView* simultaneously with the data communication links between the two programs allows the operator to accomplish this.

IMPORTANT: *TriggerSync must be started AFTER PrairieView is started! If both programs are running and you are unsure which was executed first, simply stop TriggerSync and then re-execute it prior to proceeding.*


In general the basic procedure is to enable the appropriate scan mode from within *PrairieView* then to configure the electrophysiology and photolysis parameters within *TriggerSync*, and finally to initiate data acquisition.

Following are several examples that illustrate common acquisition scenarios. The intent of this section is to provide step-by-step sequences that may be followed in order to collect various types and combinations of data. Detailed instructions on the commands listed are NOT included below. It is assumed that the operator is already familiar with the individual commands as described elsewhere in this manual.


9.1 *TriggerSync Electrophysiology Acquisition*

- Click on '**Acquisition and DAC Output Setup**'
 - Confirm or define stimulus control and acquisition parameters.
- Enable the desired ADC input channels on main *TriggerSync* window
- Press '**Acquire**' in the main *TriggerSync* window

9.2 Line scans & Electrical recording

To record with *TriggerSync* during a line scan start *TriggerSync* while *PrairieView* is running. Using *PrairieView*, choose the appropriate scan, pan, and zoom settings to position the image of the specimen. Press the Line Scan button  and position the line scan where desired. Press 'Begin Linescan'.

9.2.1 Line scanning with electrical recording & stimulation

- Start *PrairieView*.
- Start *TriggerSync*.
- Click on '**Acquisition and DAC Output Setup**' in *TriggerSync*.
 - Confirm or define stimulus control and acquisition parameters and close the dialog.
- Enable the desired ADC input channels on the main *TriggerSync* window.
- In *PrairieView* use '**Live Scan**' or '**Single Scan**' to locate the area of interest.
- Use pan, rotate, and zoom to orient the target region with the feature of interest.
- Press  to open the LineScan dialog. While the Linescan dialog is being started, the operator will be prompted as to whether the Linescan acquisition should be synchronized with *TriggerSync*. If the operator selects 'No', then standard linescan acquisitions will be performed. If the operator selects 'yes', then a *TriggerSync* acquisition will be automatically performed when the linescan acquisition is started. The *TriggerSync* data will be automatically saved in the same directory, using the same base name, as the linescan data. Position the line where desired.
- Enter the number of lines you want to collect or the amount of time to perform the linescan acquisition
- Press '**Begin Linescan**'. *TriggerSync* will perform an experiment as you have defined it while line scan data is collected.

9.2.2 Viewing Line Scan data

- Start the 'LinescanViewer' application from the Desktop.
- Load the line scan file created from the above example.
- The graph displays electrophysiology data along with fluorescence line scan profile(s).
- Create a profile.
 - Position profile in desired location by clicking in line scan window.
 - Zoom up image window if desired.
 - Copy profiles to other line scans.
 - Create multiple profiles.

- Print the graph.
- Export the data.
- 'Width' control sets number of lines to average.
- 'Move profile up/down' shifts the profile by the chosen amount.
- See the 'LinescanViewer' application documentation for a complete description of how to use this application.

9.3 Electrical recording & Uncaging

- Start *PrairieView*.
- Start *TriggerSync*.
- In *PrairieView* use '**Live Scan**' or '**Single Scan**' to locate the area of interest.
- Use pan, rotate, and zoom to orient the target region.
- Load the correct point calibration file for your scan parameters.
 - Open the Mark Points dialog in *TriggerSync*.
 - Load the appropriate calibration file for the 'Uncager Calibration File' field.
- Click on '**Acquisition and DAC Output Setup**' in *TriggerSync*.
 - Confirm or define stimulus control and acquisition parameters and close the dialog.
- Enable the desired ADC input channels on main *TriggerSync* window.
- Mark Points
 - Open the Mark Points dialog in *TriggerSync*.
 - Press 'Snap' in *TriggerSync* to collect a reference image on which to mark your points.
 - Use any combination of discrete points, lines of points, or rectangular arrays of points.

MODE 1: Run Acquisition at Each Point

(NOTE: The current cycle number and point number can be viewed in the upper right corner of the main *TriggerSync* window.)

Select 'Acquire Loop' option in main *TriggerSync* Window. This will run the 'Cycle Number' of acquisitions (set in the Acquisition and DAC Output Setup dialog) at each point of Uncaging.

MODE 2: Move to Different Points During a Single Acquisition

(NOTE: The 'Point Number' in the upper left corner of the main *TriggerSync* window does not update when collecting data in this mode.)


- Select '**Use Marked Points During Acquisition**' at the bottom of the Mark Points window in *TriggerSync*.
 - (Move time is on the order of 0.10 ms.)
 - Set first point delay time
 - Verify the 'Hold' and 'Move' times.
- Press '**Acquire**' in *TriggerSync*.

9.4 Line scans, Electrical recording, & Uncaging

- **Start** *PrairieView*.
- **Start** *TriggerSync*.
- In *PrairieView* use '**Live Scan**' or '**Single Scan**' to locate the area of interest.
- Use pan, rotate, and zoom to orient the target of the specimen.
- Load the correct point calibration file for your scan parameters.
 - Open the Mark Points dialog in *TriggerSync*.
 - Load the appropriate calibration file for the 'Uncager Calibration File' field.
- Mark Points to define locations to be uncaged.
 - Open the Mark Points dialog in *TriggerSync*.
 - Press 'Snap' in *TriggerSync* to collect a reference image on which to mark your points.
 - Use any combination of discrete points, lines of points, or rectangular arrays of points.
- Click on '**Acquisition and DAC Output Setup**' in *TriggerSync*.
 - Confirm or define stimulus control and acquisition parameters and close the dialog.
- Enable the desired ADC input channels on main *TriggerSync* window.

Move to Different Points During a Single Acquisition

(NOTE: The 'Point Number' in the upper left corner of the main *TriggerSync* window does not update when collecting data in this mode.)

- Select '**Use Marked Points During Acquisition**' at the bottom left hand corner of the Mark Points dialog window in *TriggerSync*.
 - (Move time is on the order of 0.10 – 0.20 ms.)
 - Set first point delay time
 - Verify the 'Hold' and 'Move' times
- Press  to open the Line Scan dialog. While the Linescan dialog is being started, the operator will be prompted as to whether the Linescan acquisition should be synchronized with *TriggerSync*. If the operator selects 'No', then standard linescan acquisitions will be performed. If the operator selects 'yes', then a *TriggerSync* acquisition will be automatically performed when the linescan acquisition is started. The *TriggerSync* data will be automatically saved in the same directory, using the same base name, as the linescan data Position the line where desired.
- Enter the number of lines you want to collect.
- Press '**Begin Linescan**'. *TriggerSync* will perform an experiment as you have defined it while line scan data is collected.

9.5 Non-Line Scan Images, Electrical Recording & Uncaging

- **Start *PrairieView*.**
- **Start *TriggerSync*.**
- In *PrairieView* use '**Live Scan**' or '**Single Scan**' to locate the area of interest.
- Use pan, rotate, and zoom to orient the target region.
- Load the correct point calibration file for your scan parameters.
 - Open the Mark Points dialog in *TriggerSync*.
 - Load the appropriate calibration file for the 'Uncager Calibration File' field.
- Mark Points to define locations to be uncaged.
 - Open the Mark Points dialog in *TriggerSync*.
 - Press 'Snap' in *TriggerSync* to collect a reference image on which to mark your points.
 - Use any combination of discrete points, lines of points, or rectangular arrays of points.
- Click on '**Acquisition and DAC Output Setup**' in *TriggerSync*.
 - Confirm or define stimulus control and acquisition parameters and close the dialog.
- Enable the desired ADC input channels on main *TriggerSync* window.

Move to Different Points During a Single Acquisition

(NOTE: The 'Point Number' in the upper left corner of the main *TriggerSync* window does not update when collecting data in this mode.)

- Select '**Use Marked Points During Acquisition**' at the bottom of the Mark Points dialog window in *TriggerSync*.
 - (Move time is on the order of 0.10 – 0.20 ms.)
 - Set first point delay time
 - Verify the 'Hold' and 'Move' times
- In the *TriggerSync* main window, select the menu option 'Acquisition', then select the 'Start Experiment' option, and then select the '**TTL Trigger Start**' option (this action will place a checkmark in front of this menu option indicating that it has been turned on).
- Press the '**Acquire**' button in *TriggerSync*. At this time the *TriggerSync* acquisition will begin, but it will only proceed to the point of waiting for the "trigger" signal to arrive when the start of an image collection from *PrairieView*.
- Press '**Single Scan**' in *PrairieView*. When the image scan begins, a "trigger" signal is sent to the *TriggerSync* acquisition hardware causing

TriggerSync to collect the electrical data in synchronization with the image data.

- The previous two steps must be repeated for each combined image/electrical acquisition.
- In the *TriggerSync* main window, select the menu option 'Acquisition', then select the 'Start Experiment' option, and then select the '**TTL Trigger Start**' option (*this action will remove the checkmark in front of this menu option indicating that it has been turned off*).

9.6 Functional Map Acquisition

- Start *PrairieView*.
- Start *TriggerSync*.
- In *PrairieView* use '**Live Scan**' or '**Single Scan**' to locate the area of interest.
- Use pan, rotate, and zoom to orient the target region.
- Load the correct point calibration file for your scan parameters.
 - Open the Mark Points dialog in *TriggerSync*.
 - Press 'Snap' in *TriggerSync* to collect a reference image on which to mark your points.
 - Load the appropriate calibration file for the '**Uncager Calibration File**' field.
- Mark Points to define locations to be uncaged.
 - Use the Mapping Setup section of the Mark Points dialog to create a grid of marked points.
 - To perform a Functional Map acquisition, the marked points list must only consist of a single grid of marked points as defined in the Mapping Setup procedure
- Click on '**Acquisition and DAC Output Setup**' in *TriggerSync*.
 - Confirm or define stimulus control and acquisition parameters then close the dialog.
- Enable the desired ADC input channels on main *TriggerSync* window
- Turn on the Functional Map acquisition mode by clicking on the 'Functional Mapping' checkbox along the right hand side of the main *TriggerSync* window.
- Press '**Acquire**' in *TriggerSync*.
- Save the Functional Map data.

9.6.1 Display and analysis of Functional Maps

For a description of the display and analysis options for Functional Map data, see the *TriggerSync* documentation Section [8.5.5](#).

List of Figures

Figure 3.1: Ultima and Ultima IV (<i>in vivo</i>) system without light boxes	15
Figure 3.2: Ultima System with light box and beam cover	15
Figure 4.1: Ultima electronics rack	16
Figure 4.2: Dual Preamplifier & HV control unit	17
Figure 4.3: NI BNC-2090 boxes A (top) & B (bottom)	21
Figure 4.4: Close-up of NI BNC-2090 boxes	22
Figure 4.5: NI BNC-2110	23
Figure 4.6: Ultima Scan Head light path	28
Figure 4.7: Ultima scan head dichroic locations	29
Figure 4.8: Emission Light Path for dual-channel detectors	30
Figure 4.9: Emission Light Path for quad-channel detectors	30
Figure 7.1: <i>PrairieView</i> Main Window.	37
Figure 7.2: <i>PrairieView</i> Main Screen	38
Figure 7.3: Scan resolution tools	39
Figure 7.4: Zoom and dwell time tools.	40
Figure 7.5: Scanning tool.	41
Figure 7.6: Shutter and imaging mode	42
Figure 7.7: Label and objective lens selection.	42
Figure 7.8: Pan Control & XY Stage Control	43
Figure 7.9: Scan Rotation.	43
Figure 7.10: Laser, PMT, DAQ Controls.	44
Figure 7.11: 2P Laser Control	45
Figure 7.12: Z-series setup.	46
Figure 7.13: Z-Motor control.	46
Figure 7.14: Z-series calculator	47
Figure 7.15: T-series tab.	49
Figure 7.16: XY-Stage tab.	52
Figure 7.17: Misc tab.	53
Figure 7.18: Scan information	54
Figure 7.19: The Image Window	58
Figure 7.20: Brightness Over Time windows.	61
Figure 7.21: Line Scan Definition tool.	62
Figure 7.22: Linescan Control	63
Figure 7.23: Line Scan Data display.	63
Figure 7.24: Linescan Viewer.	63
Figure 7.25: Lookup Table.	65
Figure 7.26: Define an ROI and ROI image	67
Figure 7.27: PA Masks and Acquired Z-series with masks	69
Figure 7.28: 'µm' (Line Profile Measurement) windows.	70
Figure 7.29: MIP (Maximum Intensity Projection) window.	71
Figure 7.30: Image Window and "Mark Points" window.	72
Figure 7.31: Mark Points: line.	73
Figure 7.32: Mark Points: grid	73

Figure 8.1: Main <i>TriggerSync</i> window.....	74
Figure 8.2: Analyze Acquired Data mode.	75
Figure 8.3: <i>TriggerSync</i> 'Acquire'.....	75
Figure 8.4: 'View Channel Sensitivity'.....	76
Figure 8.5: Change Scale & Auto Scale.....	76
Figure 8.6: Sequential File.....	77
Figure 8.7: Save Experiment.	77
Figure 8.8: Acquisition and DAC Output Setup.....	78
Figure 8.9: Acquisition Setup.....	79
Figure 8.10: Stimulus Control.	80
Figure 8.11: I/V Setup.....	82
Figure 8.12: Ramp.....	82
Figure 8.13: Pulse/Train.....	83
Figure 8.14: Custom Waveform.....	84
Figure 8.15: Use Marked Points During Acquisition.....	84
Figure 8.16: DAC Out Composite graph.....	86
Figure 8.17: ADC inputs.....	86
Figure 8.18: ADC Configuration.....	87
Figure 8.19: Point Calibration dialog.....	90
Figure 8.20: Mark Points window.....	98
Figure 8.21: Show map boundary.....	105
Figure 8.22: Show map points.....	108
Figure 8.23: Functional Map Display/Analysis.....	109
Figure 8.24: Functional Map example.....	110
Figure 8.25: Functional Map Calibrated Display scale.....	111

List of Tables

Table 1: Maximum NI-6052 and NI-6713 Sample Rates.....	19
Table 2: Motor Controller Resolution	25
Table 3: "Non-Neighbor" order of uncaging example.....	107