MSCAN OPTIONS / IMPORTANT TIDBITS

Date: 2020-10-28
Tags: *2P Sutter MSCAN Resonance*
Created by: Darik ONeil

IMPORTANT NOTE ON MSCAN, ANGLE OF DEFLECTION, AND FIELD OF VIEW

MSCAN has an error in the Windows 10 version of MSCAN. The X Axis Maximal Command Voltage is actually the *Y-Axis* Maximal Command Voltage.

To achieve the maximal field of view within *software* limitations, X-Axis Max Command Voltage equals [(Y-Axis Maximal Command Voltage)/(Magnification * Aspect Ratio * 0.81)]

Not even Sutter technicians know exactly what this mysterious 0.81 value is, but it is known to be related to the amount of the image tossed during turnarounds.

Back of napkin math says 512 pixels / 636 pixels (256/2; 128 pixel turnaround) is 0.80, so perhaps that's its origin. That is, number of two-channel X-Pixels / (two-channel X-Pixels + Turnaround Pixels).

IMPORTANT NOTE ON MSCAN & COMPUTER-LASER CONTROL

"Don’t use MScan control of laser. If this causes a problem let me know. As far as I know it has never worked or it worked with maybe two relatively common lasers at the time it was originally written." -- Rick Ayer, Sutter

HARDWARE

Data acquisition hardware. The workstation is equipped with three National Instruments data acquisition boards:

- One PCI-6110 S- series for scan pattern control and image acquisition.
- One PCIe-6353 X-series to control the output power of the imaging and photostimulation laser and the fine position of the objective in the z- direction.
- One PCIe-6321 X- series data acquisition board for electrophysiology.

For **resonant scanning**, the workstation features also a Measurement Computing DAS-4020/12 fast digitizer.

Included Device Drivers: NI-DAQmx; MPC-200

Tidbits

- XZ with z-piezo only for conventional scanning

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- Stacks can be automatically adjust laser power as imaging depth increases
- Arbitrary scans of points lines rectangles and ellipses/polygons only with conventional scans
- when imaging not required, and conventional scan only, photostimulation through imaging pathway of any combination of points, lines, rectangles or polygons/ellipses
- Behavioral camera hardware sync trigger

OUTPUTS

2 ANALOG

8 DIGITAL

PERIPHERALS

TTL-imaging shutter

Imaging Pockels cell. Support for an imaging Pockels cell is built in MScan. The Pockels cell is used to set the laser power output and to blank the beam during linescan returns. Setting laser power is script- compatible. We recommend using a Conoptics 350-80LA with BK (resonance-dampened) option which is optimized for multiphoton microscopy and an appropriate amplifier such as the model 302RM. Amplifiers with 50 W input only should be avoided as the data acquisition boards in the MCS computer have insufficient current output to drive these amplifiers.

Photostimulation Laser. MScan is designed to control a photostimulation laser, which can be either an ultrafast laser (to be used, for instance, in two-photon uncaging experiments) or an affordable blue/green laser diode from RGBLase. In the first case intensity is set by a dedicated Pockels cell. In the second case, the laser power is set to any value between 0 and full power directly by MScan which outputs an analog voltage directly to the laser head

Z-piezo objective positioner. MScan can perform XZ-Time frame imaging or fast XYZ-Time volumetric imaging with an optional, analog-controlled z-piezoelectric objective nanopositioner. MScan outputs a 0-10V signal to move the objective to its intended position (CONVENTIONAL ONLY)

Imaging Pockels cell. MScan is compatible with ConOptics Pockels cell amplifiers that have an input impedance of 1 KW such as the Model 302RM. Connect the driver input to the AO0 output of the first BNC-2110 breakout box connected to the PCIe-6353. Set the input range selector of the modulator to unipolar positive and to 1 KW. Amplifiers with an input impedance of 50 W only are not recommended because the data acquisition boards in the MCS computer cannot output enough current to drive these amplifiers, unless a current boosting circuit is installed between the data acquisition board and the Pockels cell amplifier. MScan can also output up to two constant bias voltages via the AO2 and AO3 analog outputs of the PCIe-6353 board (see above). These bias voltages can be set from the **Hardware** menu in MScan.

Z-PIEZO OR POCKELS CELL BIAS; conflicting recommendations

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RESONANCE SETTING

With **Resonant scanning** enabled, the **X- maximal amplitude** input box specifies the voltage applied to the resonant scanner electronics to set the maximal deflection of the fast axis mirror. The voltage range is 0-5 V, with 5 V corresponding to ~ a 26° deflection. The **Aspect ratio** input box value is used to scale the voltages sent to the slow Y-axis mirror to obtain an image that has identical dimensions on the X and the Y directions. Inversion of horizontal lines and vertical frames are possible by selecting the appropriate checkboxes in order to match the orientation of the images with that of the preparation.

The **Y-Shift** and **Turnabout** parameters optimize resonant scanning parameters. Y-Shift controls the pixel offset in the data stream coming from the device driver. If the image wobbles up and down slightly, adjusting the value in the Y-Shift input box will help. Increase or decrease this value by increments of 0.1 until wobble is minimized. Turnabout specifies the number of pixels assigned to the turnabout of the fast mirror to discard. **Y-Shift** and **Turnabout** values are preset and should be changed only with the greatest caution. **Photostimaging. These features are not available in resonant scanning configuration.**

IMAGE SETTINGS

11-bit images,

All channels must have identical gain (+10,+-5,+-2,+-1). Resonance is restricted to +-5 & +-1

Resonant scanning. The **Left Border** setting **Turnaround** sets the first displayed pixel in a resonant line scan. You will have to adjust **Left Border** for instance when changing magnification. The **Linear Correction of Sinusoidal Path** checkbox corrects for the increasing spatial distortion of line scans at the edge of the image by compressing the edge pixels towards the center. The resulting increased uniformity in spatial resolution across the field of view reduces the actual width of the image, however. This can be seen by two bands on each side of the window, filled with the Saturation color of the channel, that contain invalid pixels.

Pixel Duration: This parameter sets the pixel dwell time. The default value is 1 ms in the XY galvanometric scanning configuration. This value is fixed in resonant scanning.

Pixel Rate (Hz): The reciprocal of the Pixel Duration. MScan limits the pixel rate to be between 10 kHz and 5 MHz in XY galvanometric scanning**. In resonant scanning mode, the Pixel Rate is either 12.5 MHz when acquiring 1 or 2 channels and 6.25 MHz when sampling 4 channels.**

Frame Rate: The frame rate is calculated by taking into account the dimensions of the frame, extra pixels to include laser beam turnabout and the pixel duration. The frame rate can be entered directly by clicking on the **Edit ->** button. **In resonant scanning mode, the Frame Rate is set by the number of lines in a movie since each line takes ~ 63 ms to complete.**

Signal intensity drop-down list: Select a signal intensity compatible with the brightness of your

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preparation. This parameter sets the input range of the PCI-6110 or the PCI-DAS4020/12 imaging board. This is the same parameter as the one on the **Image Settings** page of the Viewer window. **In resonant scanning mode, the gain must be either “Very Weak Signal” or “Strong Signal” and is automatically set to the same value in all channels.**

In resonant scanning mode, frame size depends on the number of channels sampled. With 1 or 2 channels selected, the frame width is always 512 pixels. The frame height can be as small as 16 lines and up to 512

lines. When 4 channels are chosen, frames are 256 pixels wide and have a height of up to 256 lines.

In resonant scanning mode, line scan is limited to a horizontal line of either 512 or 256 pixels.

Photostimaging. These features are not available in resonant scanning configuration.

Standard Frame (up to 512 x 512): This button selects the standard frame size. In this mode, frames have a maximum number of lines (Frame Height) less than 512 and a maximum number of pixels per line (Frame Width) less than 512.

· **Frame Width**: enter the number of pixels per line. **In resonant scanning mode, the frame width is either 512 pixels when acquiring one or two channels or 256 pixels in the case of four channels.**

· **Frame Height**: enter the number of lines per frame. **In resonant scanning mode, the maximal frame height is either 512 pixels when acquiring one or two channels or 256 pixels in the case of four channels. Also, the number of lines must be a multiple of 4 with frames 512 pixels wide and a multiple of 8 when frames have a width of 256 pixels.**

· **X- offset**: the location of the leftmost pixel. **Always set to 0 in resonant scanning.**

· **Y- offset**: the location of the topmost line

· **Center Frame** (XY galvanometric scanning only): centers the frame in the 512 x 512 scanning space

Symmetric YScan check box: when unchecked, the scan pattern of the Y mirror (the slow axis by default) consists of a ramp followed by an immediate return to the location of the first line. When checked, the scan pattern is made of two symmetrical ramps. Symmetric Y Scan is preferable when fast frame rates are desired.

Magnification: a non-integer magnification factor can be entered to override the integer magnification factor set on the MOM control panel. Magnification reduces the amplitude of the scan mirror course without changing the number of

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pixels in a frame.

link : https://localhost:443/database.php?mode=view&id=22