ScanImage for *in vivo* laser scanning microscopy

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Overview

ScanImage is software to control laser scanning microscopes, with an emphasis on neuroscience applications. ScanImage is written in Matlab and was originally released in 2003 (r2.0).

Recent and upcoming releases of ScanImage are aimed at enhancing its capabilities for *in vivo* functional imaging experiments, with requirements for continuous, extended, and fast imaging in conjunction with behavioral data and/or sensory stimulation.

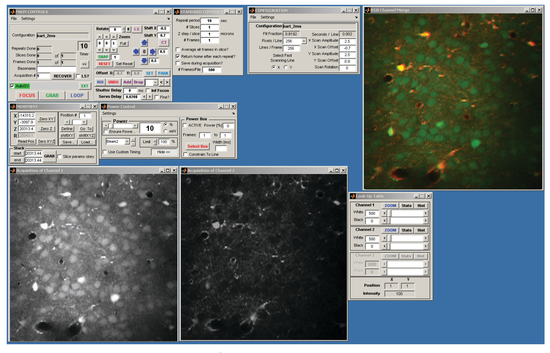
CURRENT RELEASE HIGHLIGHTS

Release 3.5 (December 2008)
- Compatible with recent Matlab versions
- Continuous gap-free data logging to TIF file
- Externally triggered acquisitions
- Live false-color channel merge display

Release 3.6 (December 2009)
- Bidirectional scanning
- Enhanced scan configuration capabilities
- Support for 4 input channels, with adjustable ranges
- Improved data processing performance, allowing high frame rates

Release 3.7 (Beta expected January 2010)
- Data Acquisition toolbox no longer required
- Further improved data processing performance
- *'Next' triggering* feature -- for data binding with external software
- Other synchronization features, e.g. exported line/frame clocks
- Automated power adjustment during Z stack collection

ScanImage 3.5



ScanImage 3.5

ScanImage 3.5 was released in December 2008

ℹ ScanImage 3.5 is very similar to ScanImage 3.0 ➔ intended for legacy users only

✅ Users new to ScanImage (<1 yr) should use ScanImage 3.6 and/or 3.7

ScanImage 3.5.1 to be available December 2009
- Several small bugfixes
- Simplified INI file in format compatible with ScanImage 3.6 & 3.7

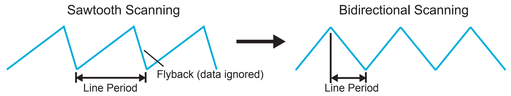
✅ ScanImage 3.5.1 smooths future upgrade to ScanImage 3.6 or 3.7

Parameter Files in ScanImage	
.INI	• *'Rig'* invariant parameters, i.e. do not vary by user or experiment • Typically one per installation, derived from *model* INI file supplied with release
.USR	• *User* invariant parameters ➔ typically one per user of installation ('rig') • Contains GUI window positions and other user preferences
.CFG	• *Configuration* parameters defining particular experimental mode • Users typically toggle among several CFG files (especially *fast configurations*)

ScanImage 3.6

BIDIRECTIONAL SCANNING

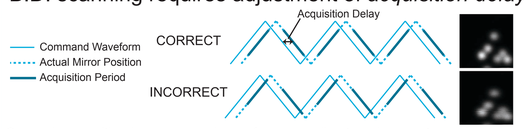
ScanImage 3.6 adds capability for *bidirectional* scanning



Bidirectional (BIDI) scanning minimizes scan bandwidth for given *line period*, allowing faster scans within galvo mechanical and servo limits.

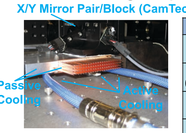
ScanImage 3.6 allows line periods down to 0.5ms/line.

BIDI scanning requires adjustment of *acquisition delay*



Scan mirror position lags command waveform according to galvanometer and servo properties. This *acquisition delay* must be correctly set to allow successive scan lines to be properly aligned.

Fast scanning requires mirror cooling



Optical Scan Range (degrees)

	15°	12.5°	10°	9°	7.5°	6.25°	5°
0.5ms/line (No Cooling)	<22 min	<22 min	<22 min	<22 min	22 min	—	39° C
0.5ms/line (Passive Cooling)	18 min	48 min	42° C	38° C	34° C	30° C	26° C
0.5ms/line (Active Cooling)	38° C	<38° C	<38° C	<38° C	<38° C	<38° C	<38° C
1ms/line (No Cooling)	39° C	<39° C	<39° C	<39° C	<39° C	<38° C	<39° C

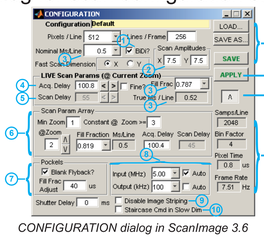
min: Time to reach 50°C ##° C: Temp at 1 hr (40-50°C) ##° C: Temp at 1 hr (< 40°C)

Galvanometers continuously scanned at fast rates over large fields require cooling to avoid temperatures causing galvo damage or wear (<50°C recommended by Cambridge Tech).

Active cooling allows continuous full field (15˚ optical range) scanning at 0.5ms/line

SCAN CONFIGURATION

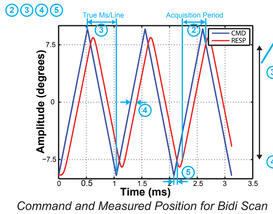
Dialog for scan configuration significantly changed in r3.6



CONFIGURATION dialog in ScanImage 3.6

① Enable *bidirectional* scanning. Selecting this adds 0.5ms/line as option for Nominal Ms/Line.

②③④⑤



Command and Measured Position for Bidi Scan (Nominal Ms/Line = 0.5ms/line; Fill Frac = 0.787)

② *Scan amplitude* (in volts) specifies range scanned within *acquisition period* (Zoom = 1) (Actual scan amplitude is larger)

③ *True Ms/Line* differs from *Nominal Ms/Line* when *Fill Fraction* ≠ 0.8192.

Key Formulae

Acquisition Period = 0.8192 × *Nominal Ms/Line*

Fill Fraction = *Acquisition Period* ÷ *True Ms/Line*

④ *Acquisition Delay* (µs) is a measured property of galvo, (mostly) independent of other properties.

⑤ *Scan Delay* (µs) represents additional time (amplitude) added to scan ramp to allow for attenuation and settling.

BIDI scans: Value determined by *Fill Fraction*

Scan Delay = (1 - *Fill Fraction*) ÷ 2

Sawtooth scans: Value, together with *Fill Fraction*, determines command amplitude and flyback period

⑥ *Scan Param Array* allows potentially amplitude-sensitive scan parameters to be optimized per zoom value. Parameters are held constant above specified zoom level. *Min Zoom* can limit scan field for configuration (e.g. to avoid thermal limits at high scan rate).

⑦ Blank power with modulator outside of *acquisition period*. *Fill Frac Adjust* adds additional time (µs) outside the *acquisition period* during which to not blank power.

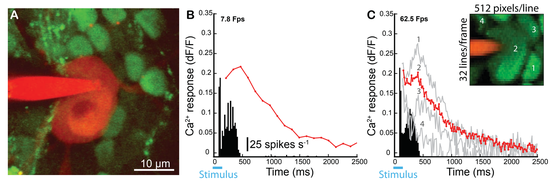
⑧ Set analog input and output digitization rates. *Auto* control links values to Nominal Ms/Line.

⑨ Disable display/processing of data in stripes (i.e. force processing per-frame). Required for cases where acquisition delay 'wraps' acquisition period to succeeding command scan line.

⑩ Use staircase (i.e. step-wise) scan command for slow scan dimension, rather than slow ramp.

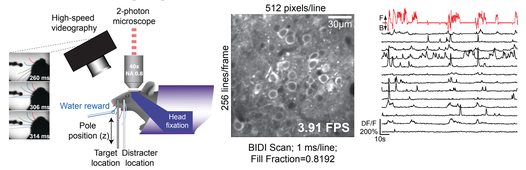
SAMPLE APPLICATIONS

Stimulus evoked calcium responses recorded at > 60 fps



(A) Retinal ganglion cells loaded with OGB-1 (green). Single brisk-transient type cell targeted for extracellular spike recording (red; Alexa 568). **(B)** Spike response and fluorescence signal measured from targeted (red) cell. Optical recording obtained at 7.8 fps (512x128 pixels; 1ms/line). **(C)** Spike response and fluorescence signals from multiple identified cells in ROI. Optical recording obtained at 62.5 fps (512x32pixels; 0.5ms/line). Bimodal spike onset distribution apparent in electrical recording is now also evident in optical recording.

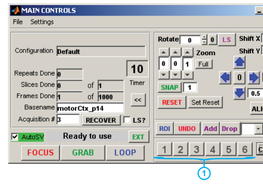
Functional imaging combined with behavior



ScanImage is increasingly employed in combination with behavioral and/or sensory stimulation apparatus/software, such as the mouse whisker stimulation/tracking apparatus depicted (left). Optical recordings at 3.9 fps (right) are obtained from identified motor cortex neurons expressing GCaMP 3.0 (center). Red trace indicates mouse running motion on treadmill.
(Tian *et al.*; *Nature Neuroscience*; in press).

OTHER ENHANCEMENTS

Fast Configuration Switching

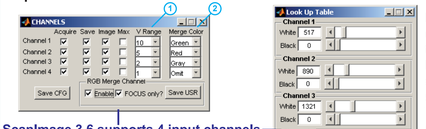


① Ready access to a set of pre-specified ('fast') configurations is now available from the **MAIN CONTROLS** dialog. Fast configurations can also be loaded by using the F1-F6 keys (as introduced in ScanImage 3.5).

② Show/hide **CONFIGURATION** dialog

Frequent scan configuration changes are expected/encouraged in ScanImage 3.6

Input Channel Enhancements



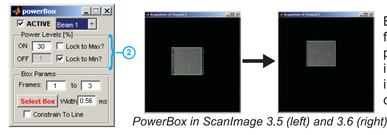
ScanImage 3.6 supports 4 input channels

① Voltage range over which to digitize input signals. Maximizes use of dynamic range.

② Identify color for each channel in live channel merge display (introduced in r3.5)

③ Interactive image tools (e.g. digital zoom, tooltips, statistics) and PowerBox/ROI selection are targeted to channel specified as Default Target OR, if 'None' is specified, user must select (click) on target channel window first.

Power Box & ROI Positioning Improvements



PowerBox in ScanImage 3.5 (left) and 3.6 (right)

Both the PowerBox and ROI features have improved positioning accuracy in r3.6, in part due to support for increased output signal digitization rate.

① PowerBox feature power levels can now be set independently of main power levels, including OFF level (power outside box)

② PowerBox ON/OFF level (power inside/outside box) can be tied to main power level via *Lock to Max/Min*. This was *only* behavior in r3.5.

ScanImage 3.7

Matlab DAQ Toolbox is no longer required

DAQ Toolbox	DAQmx Interface Package
• Supports buffered Analog Input/Output • NO support for buffered Digital Input/Output • NO support for counter/timer functionality • Raw (fast) data read operation NOT supported	• Object-oriented interface to NI DAQmx library • Comprehensive support of DAQmx capabilities • Fast raw data read operations supported

ScanImage 3.6 and earlier releases use the Matlab *Data Acquisition Toolbox* (Mathworks) for interfacing to data acquisition (DAQ) drivers from National Instruments (NI). ScanImage 3.7 employs an object-oriented *package* of *classes* that directly interface to NI's DAQmx driver.

Data processing performance improvements

	Read	Process	Wrap Fix	Display	Write	TOTAL
ScanImage 3.6 (Matlab 2007b)	2.9	2.2	1.0	4.4	0.9	13 ms
ScanImage 3.7 (Matlab 2009b)	0.6	1.8	1.0	1.9	0.8	9 ms

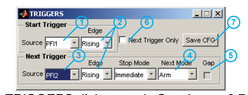
Read: Access data from DAQ driver buffer
Process: Convert data into binned image stripe/frame
Wrap Fix: Circular shift operation req'd when acquired data 'wraps' to next line
Display: Update channel display windows
Write: Append frame to open TIF file stream

Benchmark data of elapsed times (ms) for key operations on each acquired frame of data (512x32 pixels; 0.5ms/line; 2 channels). Total processing time must not exceed frame period (16ms) to sustain data stream. Green denotes ScanImage code improvements added in version.

ScanImage 3.7 continues improvements to data processing times begun in ScanImage 3.6, allowing higher frame rates and more time for future features and/or custom *user functions*.

Support for 'Next Triggering'

'Next' triggers allow ScanImage image files to be aligned in time with experimental events, e.g. behavioral cues. In example scheme shown, Next triggers simply open new TIF file stream, without interrupting acquisition.

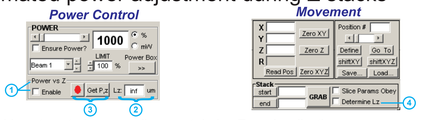


TRIGGERS dialog new in ScanImage 3.7

① Next trigger can be selected from available sources (configured in INI file)
② *Rising* or *Falling* edge can be used for Start or Next trigger.
③ Next trigger can be selected from available sources (configured in INI file)
④ Next trigger can either *Arm* next acquisition (stopping to wait for a new Start trigger) or *Advance* to next acquisition automatically.
⑤ For *Advance* mode, *Gap* mode causes acquisition to stop and restart, using internal trigger. Otherwise, acquisition is continuous (gap-free) -- next triggers simply start new TIF file stream.
⑥ *Next Trigger Only* mode regards first Next trigger as Start trigger (*Advance* mode req'd)
⑦ All **TRIGGERS** dialog parameters are saved/loaded as part of scan configuration (CFG file)

ScanImage 3.7 is aimed at enhancing coordination of imaging with behavioral and sensory apparatus/software often used during *in vivo* neuroimaging experiments

Automated power adjustment during Z stacks



① Enable automated power adjustment during Z stack collection
② Exponential decay constant L_Z used for auto-adjustment
③ 'Record' mode allows L_Z value to be determined by adjusting and recording P at several z planes
④ L_Z value can also be determined by adjusting/recording power level at stack start/end planes

Download Info

ScanImage is available for download on a wiki site:

http://openwiki.janelia.org/wiki/display/ephus

Registration is required. Since Summer 2008, over 100 labs worldwide (15 countries) have registered for *ScanImage* or *Ephus* -- a companion software package for general data acquisition [SFN 390.20].

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