ScanImage and Ephus:
Modular control software for laser-scanning and neurophysiological measurements

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Overview

Neurophysiology experiments often integrate multiple types of measurements, such as two-photon laser scanning microscopy (TPLSM), laser-scanning photostimulation (LSPS) circuit mapping, and electrophysiology. There is an increasing need for software to coordinate and automate these measurements. Such software needs to meet multiple, sometimes conflicting demands: while it should provide intuitive graphical user interfaces for ease of use, it must also match the workflow of a large variety of experiments even within a single laboratory, requiring strategies to customize the function and the 'look and feel' of the application. We present recent progress in the development of two freely available, open-source software packages, ***ScanImage*** and ***Ephus*** both written largely in MATLAB, and designed for neurophysiology laboratories.

To obtain *ScanImage* and/or *Ephus:*

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

(Registration required)

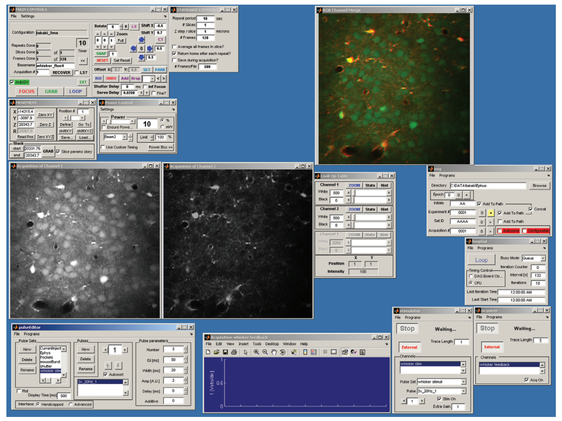
UPCOMING RELEASES

ScanImage v3.5 prerelease available *NOW*

ScanImage v3.6 prerelease anticipated ~Dec 15
(seeking beta testers *NOW*)

Ephus v2.0 prerelease available ~Dec 1

Note: *Prereleases*, unlike betas, are versions believed to be release-ready



ScanImage 3.5 (w/ basic Ephus configuration)

ScanImage is software to control laser scanning microscopes, with an emphasis on neuroscience applications. It was originally released in 2003 (v2.0), with v3.0 following in 2004, and has >1000 registered users. This year, two new versions (v3.5 and v3.6) add new features to support functional imaging applications.

ScanImage capabilities include laser scanning with analog servo-controlled galvos (e.g. Cambridge Tech), analog power modulation (e.g. Pockels Cells), and X/Y & Z motor control of the specimen or objective (Sutter MP-285).

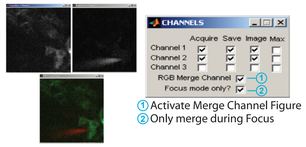
ScanImage

Important New Features in v3.5

- Compatible with recent Matlab versions (r2007b) and NI DAQmx driver

Live RGB Channel Merge

Many neurobiology applications benefit from live visualization of fluorescence from multiple indicators. Scanimage 3.5 can merge data from up to 3 input channels, and display a 'live' RGB image.



①Activate Merge Channel Figure
②Only merge during Focus

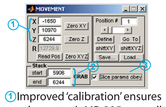
Unlimited & Uninterrupted Acquisition

In ScanImage 3.0, data collected during a GRAB or single LOOP iteration was buffered, and then saved to a file. This required 'dead' time between acquisitions and limited the maximum acquisition time. ScanImage 3.5 can stream data from up to 3 channels to disk, allowing uninterrupted acquisitions of unlimited duration.

①Enable disk logging during GRAB
②Logged data can be split into multiple files, or combined in one.

Improved MP-285 Interface

Simplified specification of MP-285 'type' eliminates need for X/Y/Z 'calibration' required in ScanImage 3.0, ensuring that X/Y/Z values and step sizes in ScanImage match values on MP-285 controller. Stack endpoints are displayed in ScanImage 3.5, and can constrain slice parameters (# slices and step size).



①Improved 'calibration' ensures values match MP-285 controller
②Start/stop endpoints displayed
③Endpoints constrain slice params

Externally Triggered Acquisitions

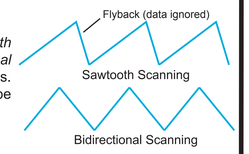
Multiphoton imaging is often done in combination with third-party electrophysiological and/or behavioral apparatus. In ScanImage 3.6, GRAB/LOOP acquisitions can be triggered by external software.

①Data-logging (or autosave) status
②Toggle use of external triggering; otherwise 'self'-triggering occurs.

Coming Soon in v3.6

Bidirectional Scanning

ScanImage 3.0 supported exclusively *sawtooth* scanning. ScanImage 3.6 adds support for *bidirectional* scanning, in which data is acquired in both directions. Bidirectional scannng allows higher line rates to be reached, within the mirror & servo's bandwidth.

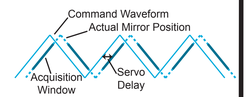


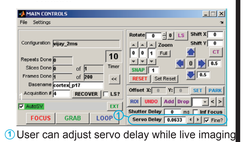
Live Servo Delay Adjustment

Galvo servo controllers delay the command signal to provide stable control. This 'servo delay' (termed 'cusp delay' in v3.0) must be known in order to acquire data at the right time (location).

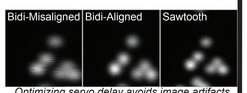
ScanImage 3.6 allows the servo delay to be optimized from the main control window, while observing a live acquired image. For bidirectional scanning, the user can then visually 'align' the data from every other line.

Ideally delay would be frequency & amplitude independent, but may not be for higher scan speeds or amplitudes. Fortunately, the delay is quite repeatable for given scan parameters.





①User can adjust servo delay while live imaging

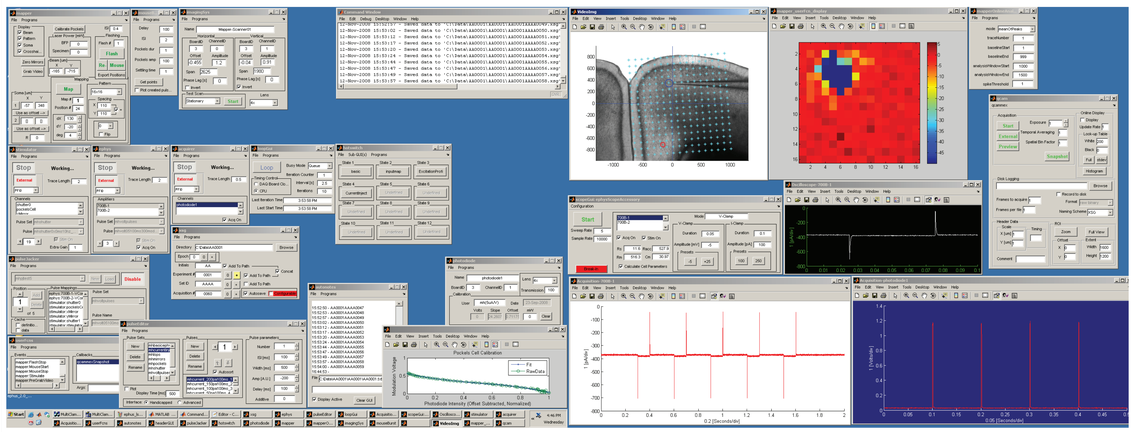


Optimizing servo delay avoids image artifacts when imaging 1μm fluorescent beads.

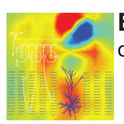
Sawtooth Scan Simplification

In ScanImage 3.0, the user adjusts three parameters for sawtooth scans: *fill fraction*, *line delay*, and *servo delay*. ScanImage 3.6 eliminates the line delay parameter and automatically optimizes fill fraction for each scan amplitude & speed. The optimum maximizes the fill fraction while avoiding image 'reflection' artifacts.

Ephus



Ephus used for Laser Scanning Photostimulation (LSPS) and standard Electrophysiology



Ephus is a highly modular collection of programs for data acquisition. It has been used in many types of electrophysiological and data acquisition applications. For example:

Laser scanning photostimulation (LSPS)-based neural circuit mapping (glutamate uncaging and ChR2 photostimulation).

Multi-channel whole-cell recording in brain slices and *in vivo*.

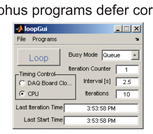
In vivo optical microstimulation mapping with CCD imaging.

Program Configurations

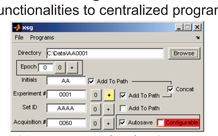
All Ephus programs are associated with configurations comprising the program properties (including location & visibility) which can be saved and loaded individually or en masse (configuration set). The Hotswitch program allows one to rapidly switch between experimental modalities in one click.

Synchronization & Data Binding

Ephus programs defer core functionalities to centralized programs.



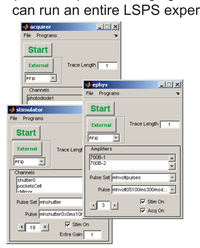
loopGui - Synchronizes & schedules program actions.



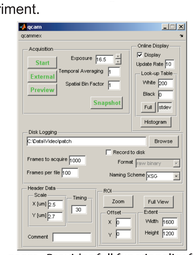
XSG - Aggregates headers (program properties) and acquired data from all programs into a shared file.

Hardware Support

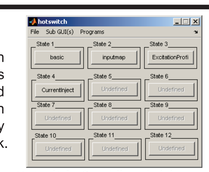
Ephus supports A/D, D/A, and DIO channels (Nat Inst), amplifiers (Axon Inst) and QImaging CCD cameras. A single NI USB board can run an entire LSPS experiment.



acquirer, stimulator, ephys - Core programs for I/O channels.



qcam - Provides full functionality for QImaging cameras within Ephus.

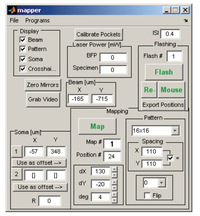


Hotswitch - Ravpidly switch between user-defined program configurations.

Extensibility

Ephus programs fire *events* to which custom *user functions* can respond.

userFcns - Binds events and user functions.



mapper - Specialized program for LSPS mapping; makes use of core programs and user functions.

Outlook

Although *ScanImage* has been widely used in the neuroscience community, emerging applications will require new modules. Currently in the works:

- Support for fast laser scanning devices (resonant galvanometers and acousto-optic deflectors).
- Support for digital galvanometer servos.
- Concurrent piezo-based fast Z-scanning.
- Development of a high-speed, variable bit-depth stream API, for more powerful data-logging.
- Tighter integration between ScanImage and Ephus.

Ephus is a powerful toolbox for data acquisition and instrument control. Currently in the works:

- Develop an Ephus Installer to streamline hardware and application configuration.
- Improved Wiki-based documentation.
- Support for additional CCD camera vendors.

Other Ideas/Requests?

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