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FFF73

ScanImage, software for *in vivo* laser scanning microscopy

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Overview

ScanImage is software to control laser scanning microscopes, particularly *two-photon microscopy* for *neuroscience* applications. ScanImage is written primarily in Matlab, with portions in C. The first public release (r2.0) was in 2003.

Recent and planned ScanImage releases aim to enhance its capabilities for *in vivo* functional imaging experiments, with requirements for fast (video rate) continuous imaging in volumes of neural tissue, synchronized in time with behavioral data, sensory stimulation, and/or electrophysiology.

RELEASE HIGHLIGHTS

Release 3.8

- Cycle mode -- timed acquisition sequences with motor operations and/or configuration changes at each *iteration*
- Enhanced point/line/square/rectangle ROI operation
- Enhanced user function capabilities, including USR-file bindings
- Streamlined graphical user interface
- Support for dual stage controller operation, e.g. XY & Z
- Operation under 64-bit Windows 7

Release 4.1

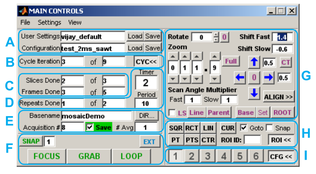
- Fast raster scanning, using resonant scanning hardware
- Fast axial scanning, using piezo actuators, synced to frame rate
- Optimized digitizer sample averaging at each pixel (in hardware)
- Power modulation, synced to line rate, with depth adjustment
- Support for long, continuous acquisitions, e.g. with *next triggering*
- Support for rectangular area and line scans
- Display of rolling average and/or selected frames/slices

User Interface Enhancements

ScanImage 3.8 & 4.0 share similar, streamlined interfaces

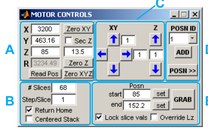
Standard/Acquisition Controls from ScanImage 3.5-3.7 ***eliminated***

Main Controls (enhanced)



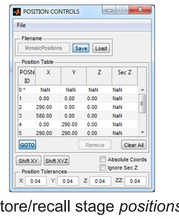
- **A** Load, save, display current User & Configuration files
- **B** Cycle Mode status; access Cycle Controls (see ScanImage 3.8)
- **C** Acquisition length control and display -- number of Slices and Frames
- **D** Loop acquisition status and controls -- number and interval of Repeats
- **E** File saving controls -- enable saving, set file name and directory, configure frame averaging
- **F** Acquisition start controls -- Focus, Grab, Loop, Snapshot modes and external start triggering
- **G** Control ROI Scan Parameters -- Zoom, Shift, Rotation, and Angle Multipliers (aspect ratio)
- **H** Graphical selection of ROIs; access ROI Controls (see ScanImage 3.8)
- **I** Load cached Fast Configuration settings; access Configuration Controls

Motor Controls (enhanced)



- **A** Set/read position of primary and *secondary* stage controller
- **B** Stack acquisition controls
- **C** Stage panning controls
- **D** Access Position Controls
- **E** Interactive specification of stack start & end points

Position Controls (new)

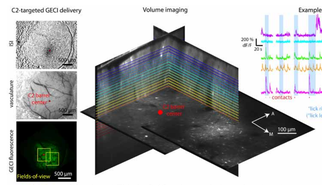


- Store/recall stage *positions*
- Positions coordinated with ROIs

Sample Applications

Imaging activity of >1000 neurons simultaneously in behaving mice

Simon Peron...Karel Svoboda (HHMI/JFRC)



Volume imaging at 7Hz in 8-16 volumes in barrel cortex, to map responses of ~10,000 neurons across multiple columns

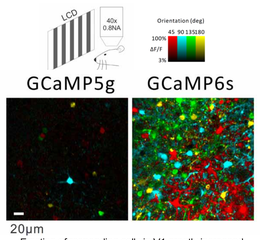
Touch responses mainly in principal whisker column; whisking responses distributed across columns

Representation of whisker touch in barrel cortex mapped in ~10,000 neurons throughout principal whisker column and neighboring columns

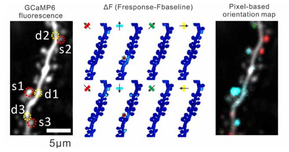
ScanImage 4.0
677.12

***Mapping receptive fields of neurons and synapses* in vivo**

Tsai-wen Chen...Doug Kim (HHMI/JFRC)



Fraction of responding cells in V1 greatly increased

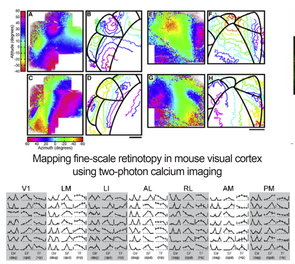


Enhanced GECI family (GCaMP6) allows single spike detection in somata and reveals orientation-selective domains in dendrites & spines

927.08 ScanImage 4.0

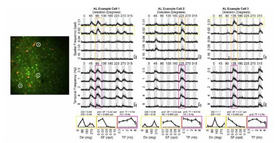
Measuring activity across different cortical areas

James Marshel...Ed Callaway *Neuron* 2011 (UCSD)



Mapping fine-scale retinotopy in mouse visual cortex using two-photon calcium imaging

Representative tuning curves from 7 visual cortical areas



Measuring selectivity for direction, spatial frequency, and temporal frequency in visual cortex anterolateral area (AL)

Fine-scale continuous retinotopy and distinct encoding of spatiotemporal features in distinct visual cortical areas mapped by two-photon calcium imaging

ScanImage 3.6

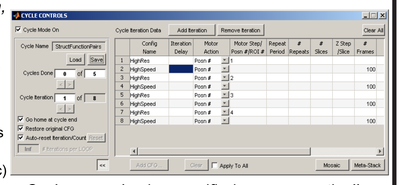
ScanImage *supports a wide range of imaging applications in living neural tissue*

ScanImage 3.8

Cycle mode allows control/timing of acquisition sequences

Before each Cycle *iteration*, can do one or more of:

- Load a Configuration file
- Wait specified *iteration delay*
- Motor action (step or go-to identified Position or ROI)
- Override configuration values with iteration-specific values (e.g. # Repeats, # Frames, etc)



Cycles can also be specified programmatically, and stored/loaded via CYC files

ROI comprised of scan parameters and stage position

ROI Scan Parameters

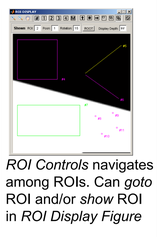
1) Zoom
2) Shifts
3) Rotation
4) Scan Angle Multipliers

Scan Angle Multiplier parameters allow for rectangle, line, and point ROIs

Most recent line ROI, and its parent area ROI, cached to allow rapid recall

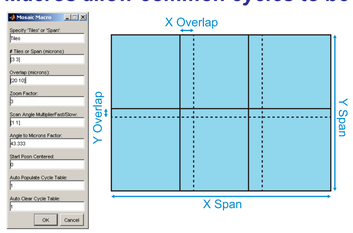
ROIs are or descend from 'top-level' area ROI with unique (Position ID, Rotation) pair

Area ROIs can have child ROIs (area or point/line)



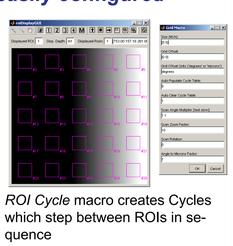
ROI Controls navigates among ROIs. Can *goto* ROI and/or *show* ROI in *ROI Display Figure*

Macros allow common cycles to be easily configured



Mosaic macro creates tiled array of *positions* with specified span and overlap

Macro can also auto-populate Cycle iterations to step between tiled positions in sequence



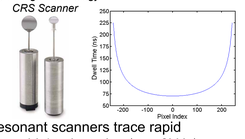
ROI Cycle macro creates Cycles which step between ROIs in sequence

Grid macro creates array of square, line, or point ROIs in a field-of-view

ScanImage 4.1

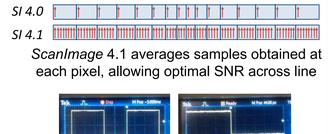
Resonant scanning - fast, non-uniform pixel dwell times

Cambridge Technology CRS Scanner

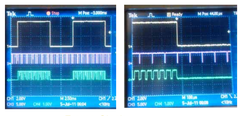


Resonant scanners trace rapid sinusoidal trajectories (e.g. 8kHz)

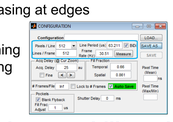
Dwell time per pixel varies across line scan, rapidly increasing at edges



ScanImage 4.1 averages samples obtained at each pixel, allowing optimal SNR across line



Bidirectional scanning allows 30Hz imaging at 512x512 pixels



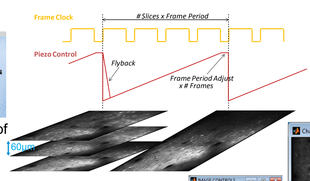
Frame Clock
Line Clock
Pockels Control

ScanImage 4.1 modulates Pockels to limit portion of line period illuminated (*fill fraction*), preventing overexposure at line edges

Volume imaging capability, with piezo-driven stage

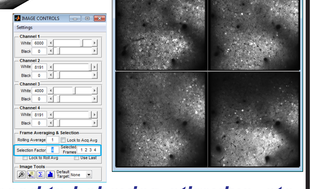
Physik Instrumente P-725 PIFOC

Piezo actuation of objective lenses achieves > 30Hz bandwidth



Fast Z scanning sweeps axial position over integer multiple of frame periods

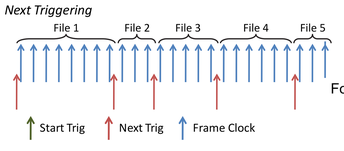
Display *frame selection* allows multiple slices to be visualized 'simultaneously'



Long acquisitions can be synced to behavior, stimulus, etc

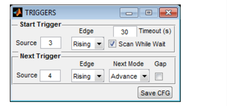
StartTriggering (Self or External)

Next Triggering



Start triggers synchronize frame series start to external event

Next triggers can *advance* file counter during continuous acquisition



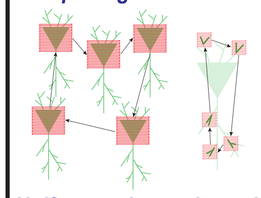
For each file, 2 timestamps saved:

triggerStartTime
Time of start/next trigger initiating file

triggerFrameStartTime
Time first imaged frame in file started

Future Plans

Multiple region-of-interest scanning

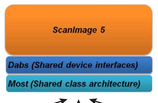


Combine resonant scanner with galvo scanner pair for multi-ROI imaging/stimulation

Advantages

- Feasible ROI selection (vs 100s of points)
- Motion tolerance
- Distributed illumination/photoexcitation

Unify experimental capabilities into common framework



Unify experimental modes into common framework with:

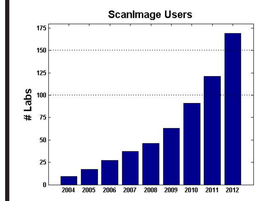
- Shared configuration files
- Shared files/filenames for bound data streams
- Shared metadata format
- Synchronized data & timestamps
- Shared scripting language
- Shared user function/plugin concepts

ScanImage 3.x (Galvo scanning) **ScanImage 4.x** (Resonant scanning) **Ephus 2.x** (Physiology, Camera Imaging, General Data Acq)

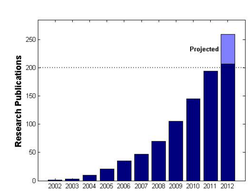
Distribution

ScanImage, and *Ephus,* are freely available at

http://scanimage.org
http://ephus.org



ScanImage used in >160 labs through 2012



ScanImage/Ephus used in >200 publications to date

- ScanImage/Ephus used in over 20 countries and over 20 US states
- About 95% of labs involved in neuroscience-related research

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