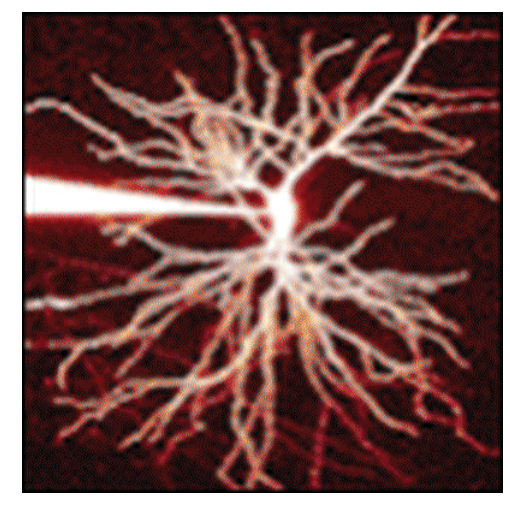# *ScanImage*, software for *in vivo* laser scanning microscopy

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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 3.7.1
● Small enhancements and bugfixes
● Suited for existing users

Release 3.8 (Release Candidate)
● Cycle mode -- timed acquisition sequences with motor operations and/or configuration changes at each *iteration*
● Enhanced point/line/square/rectangle ROI operation
● Streamlined graphical user interface
● Support for dual stage controller operation, e.g. XY & Z
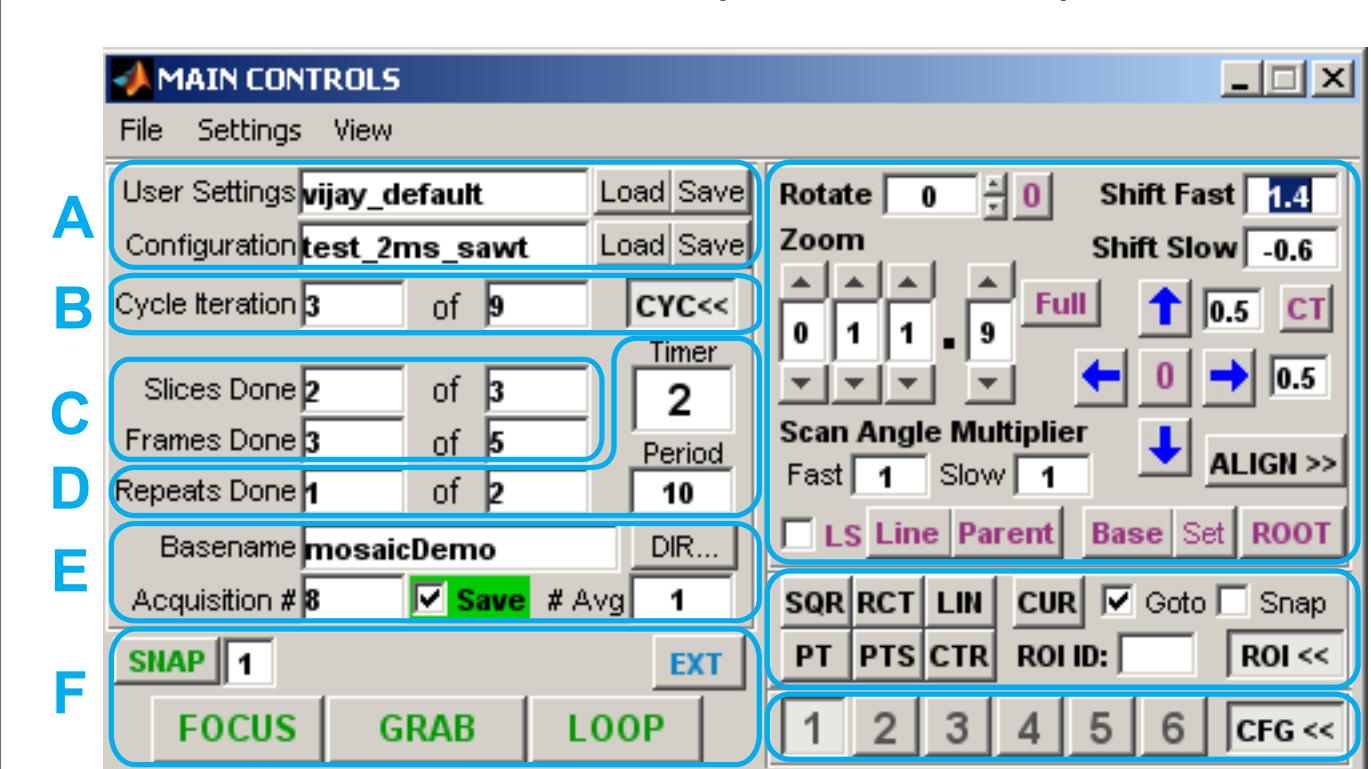● Operation under 64-bit Windows 7

Release 4.0
● Fast raster scanning, using resonant scanning hardware
● Fast axial scanning, using piezo actuators, synced to frame rate
● Power modulation, synced to line rate, with depth adjustment
● Support for long, continuous acquisitions, e.g. with *next triggering*
● Display of rolling average and/or selected frames/slices

Sample Applications

Activity in sensorimotor axons correlated to behavior
Leopoldo Petreanu, Diego Gutnisky, et al. (HHMI/JFRC)

Sensorimotor integration during mouse whisking behavior studied by imaging calcium transients in axons projecting to layer 1 of somatosensory cortex

ScanImage 3.7
704.21

Screening GECIs in visual cortex
Tsai-wen Chen...Doug Kim (HHMI/JFRC)

Highly selective responses of neurons in layer 2/3 of mouse visual cortex to drifting gratings used to assay new genetically encoded calcium indicator (GECI) candidates

(Kerlin, Reid 2010 Neuron)

204.24
ScanImage 3.7/4.0

Axonal calcium transients in cerebellar interneurons
Jason Christie, Delia Chiu, and Craig Jahr *Nature Neurosci.* 2011 (OHSU)
Christie and Jahr, *Neuron* 2008

Calcium recordings in axons of cerebellar interneurons show presynaptic plasticity arises from subthreshold somatic depolarization

ScanImage 3.5

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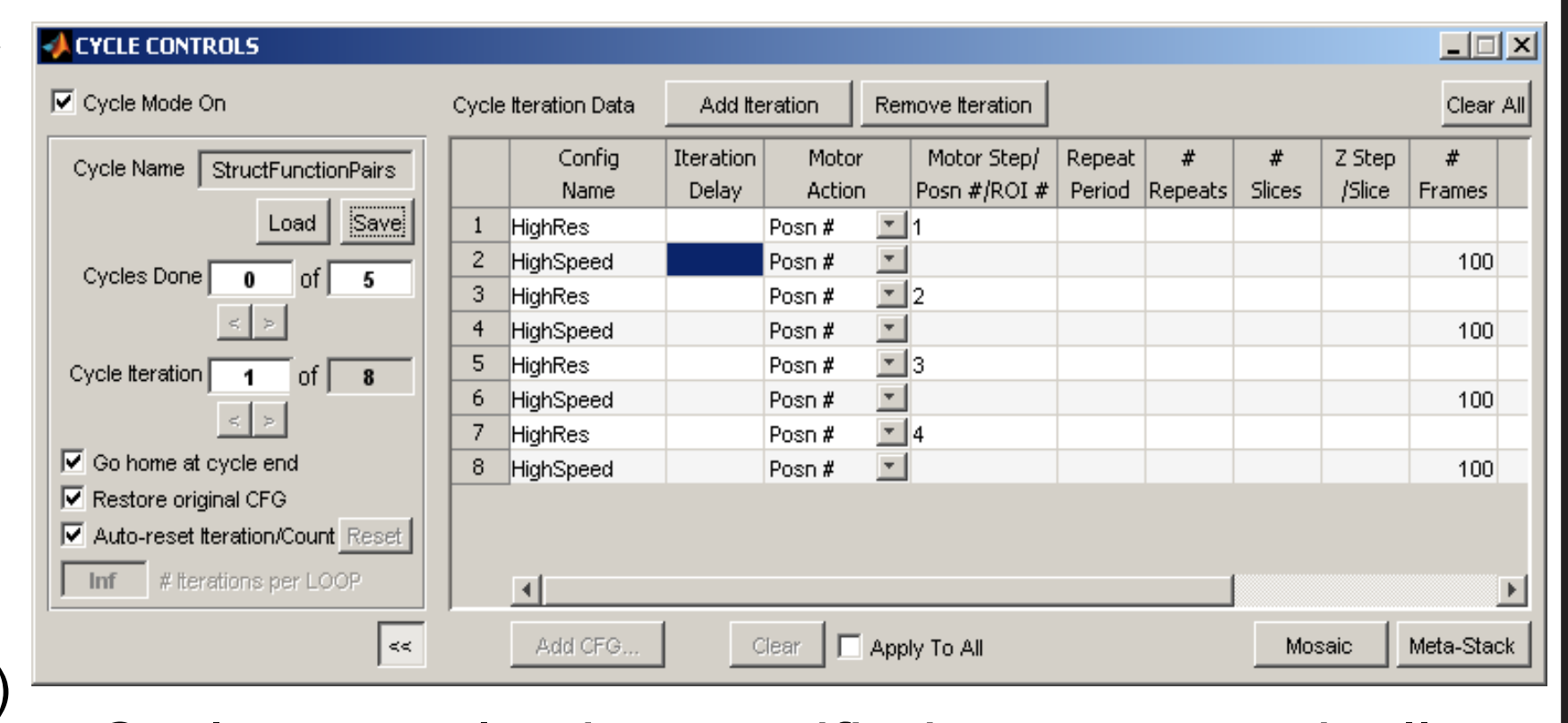
Cycles can also be specified programmatically, and stored/loaded via CYC files

ROI comprised of Scan Parameters and stage Position

ROI Scan Parameters
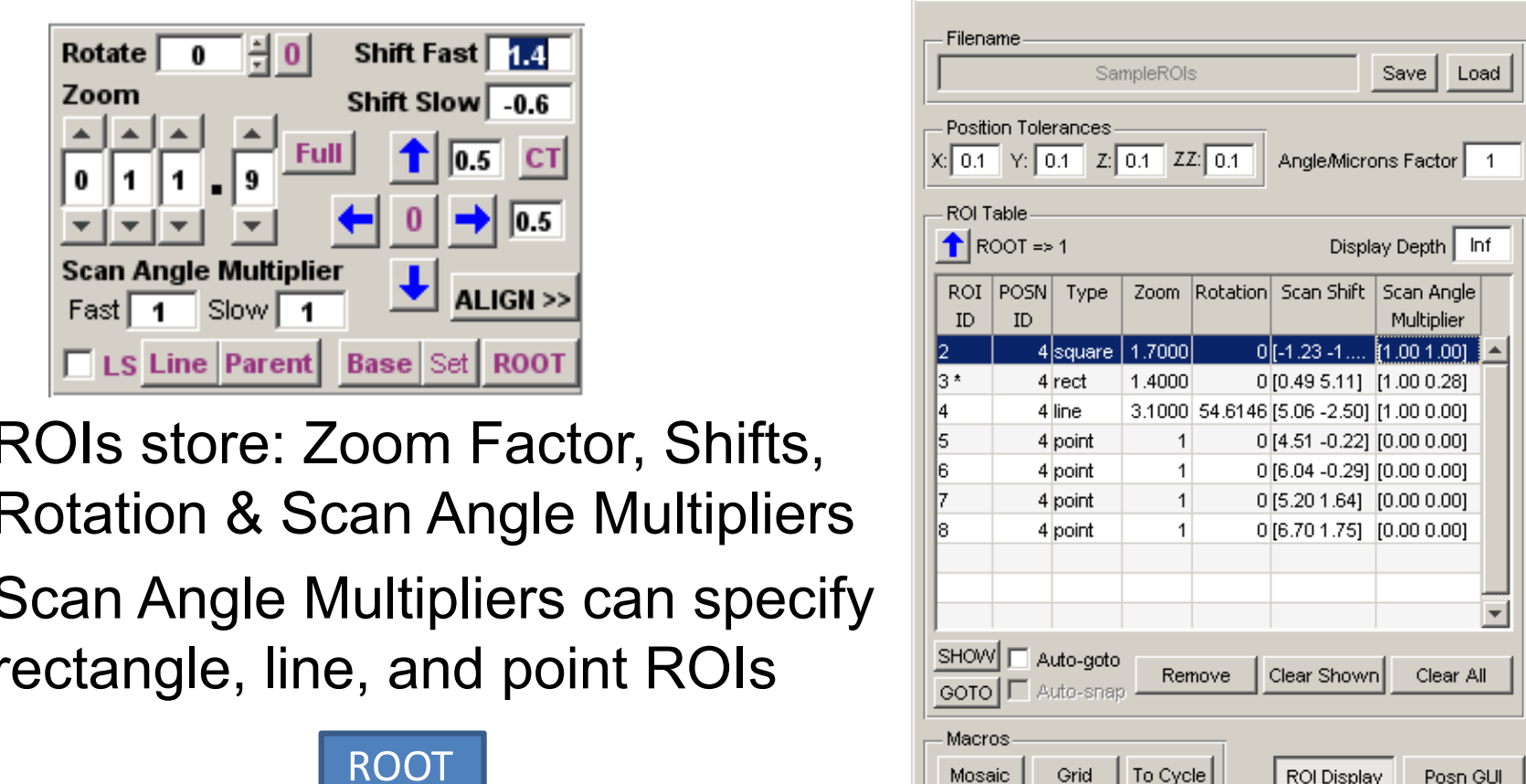
ROIs store: Zoom Factor, Shifts, Rotation & Scan Angle Multipliers
Scan Angle Multipliers can specify rectangle, line, and point ROIs

ROI Controls allow navigation and selection of ROIs to go-to or show in ROI Display Figure

All ROIs have associated stage *Position ID*, tied to 'top-level' area-type ROI

Area type ROIs can have child ROIs (area or point/line)
Most recent line ROI, and its parent area ROI, stored to allow easy switching back-and-forth

Macros allow common Cycles to be easily configured

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

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

ROI Cycle macro creates Cycles which step between ROIs in sequence

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

ScanImage 4.0

Fast raster scanning supported, with power modulation

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

With bidirectional scans, 30Hz frame rate reached with 512 x 512 pixels

Fill fraction limits portion of line period illuminated, avoiding 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

Advance mode

Arm mode

Start trigger synchronize frame series start to external event

Next triggers can stop ongoing (*arm next*) acquisition or *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

Start Trig | Next Trig | Frame Clock

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

ScanImage 5 | Ephus 3
Dabs (Shared device interfaces)
Most (Shared class architecture)

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

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

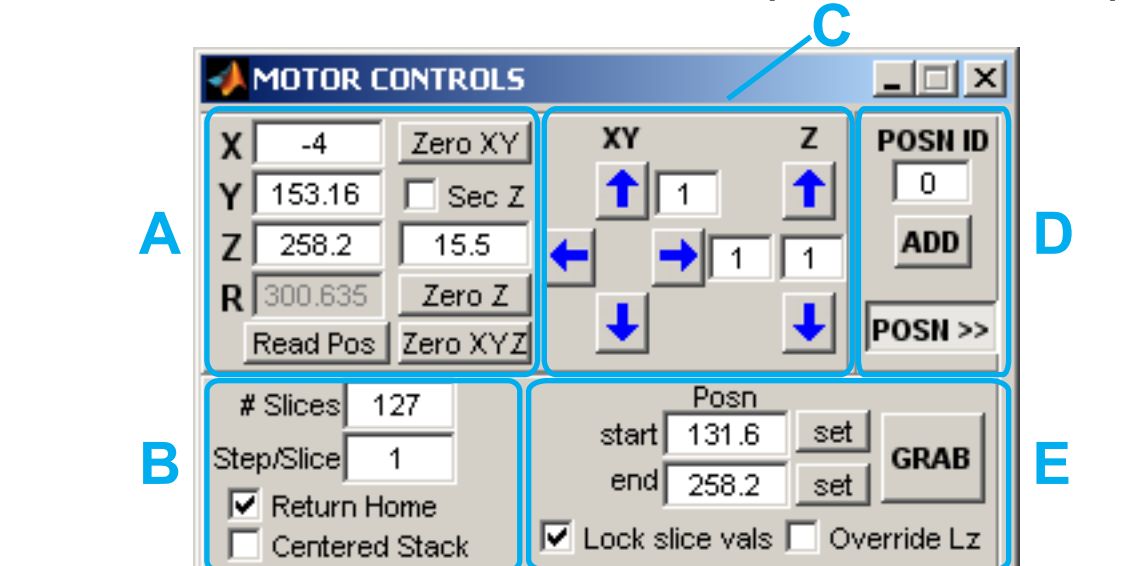## User Interface Enhancements

ScanImage 3.8 & 4.0 share similar, streamlined interface

Standard/Acquisition Controls from ScanImage 3.5-3.7 *eliminated*
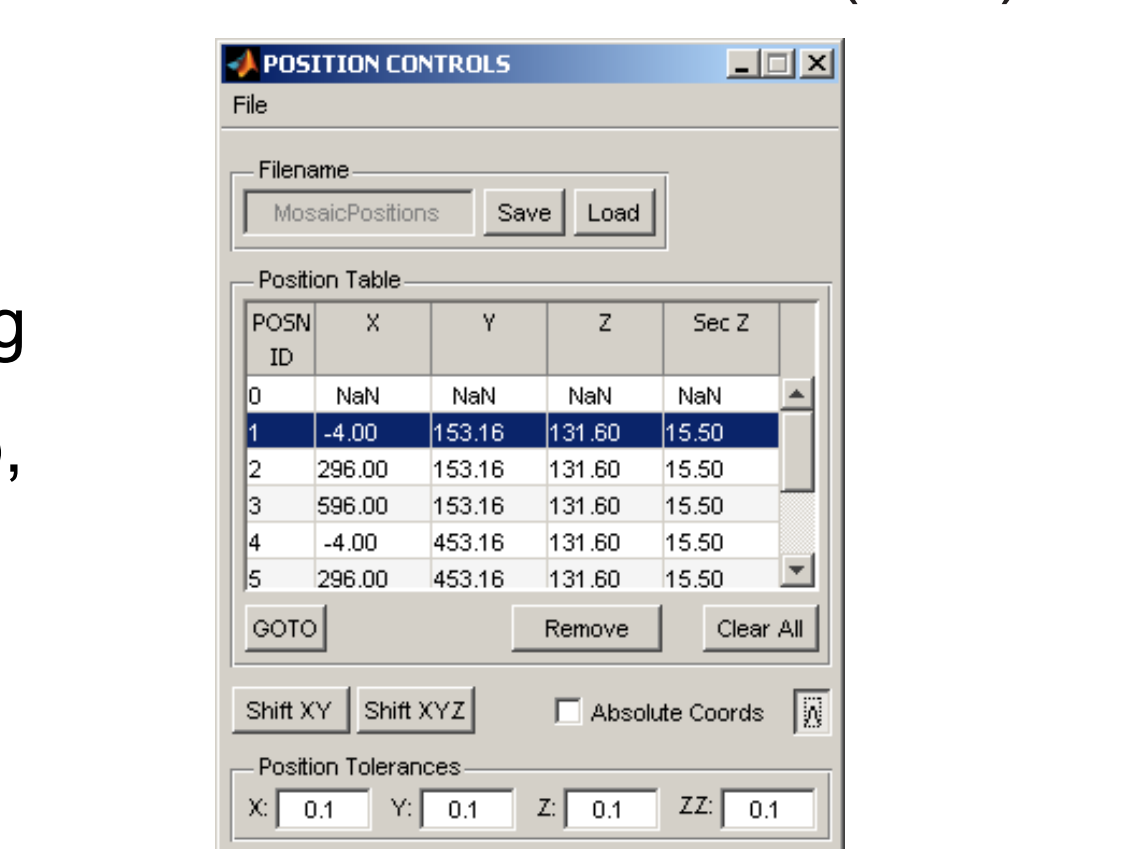
Main Controls (enhanced)   **Motor Controls** (enhanced)

A Load, save, display current User & Configuration files

B Cycle Mode status and control access (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 and access ROI Controls (see ScanImage 3.8)

I Load cached Fast Configuration settings; access Configuration Controls

A Set/read position of primary and *secondary* stage controller

B Stack acquisition controls

C Stage panning controls

D Access new Position Controls

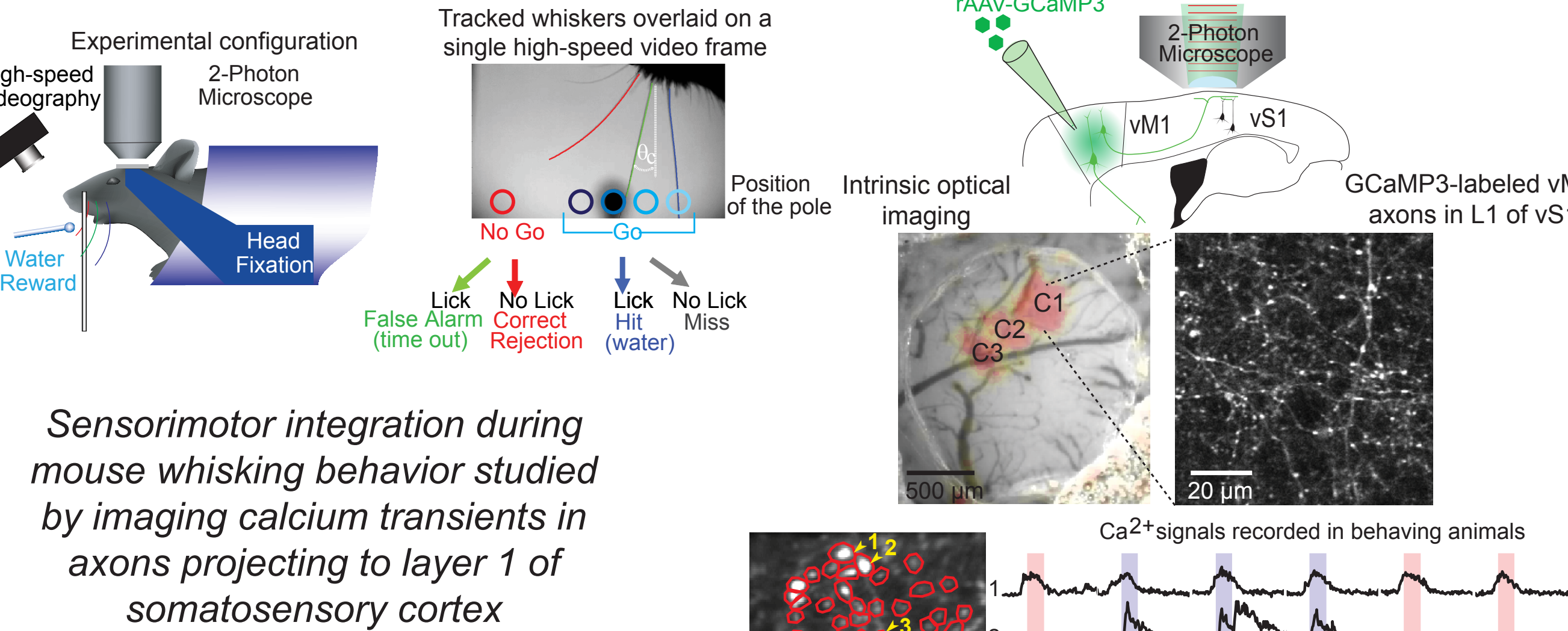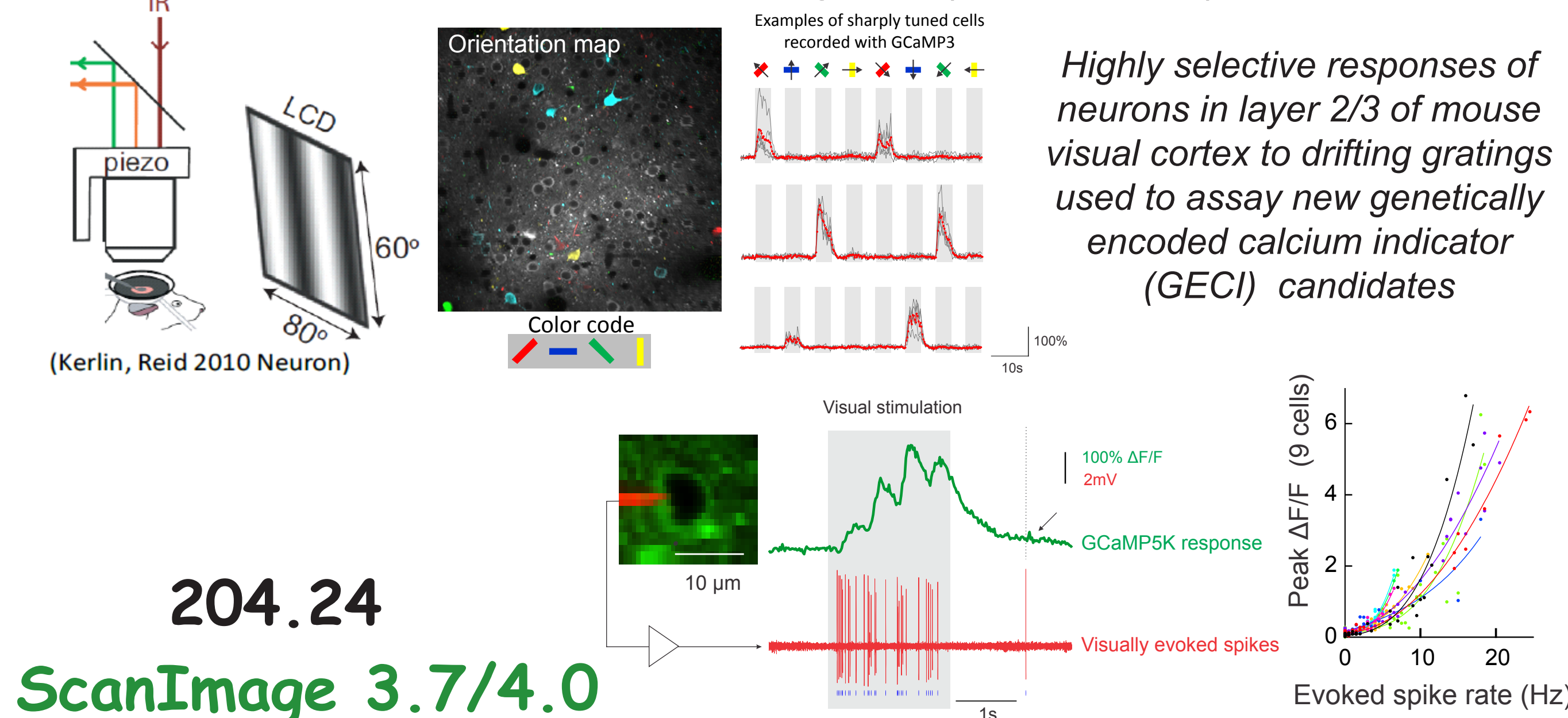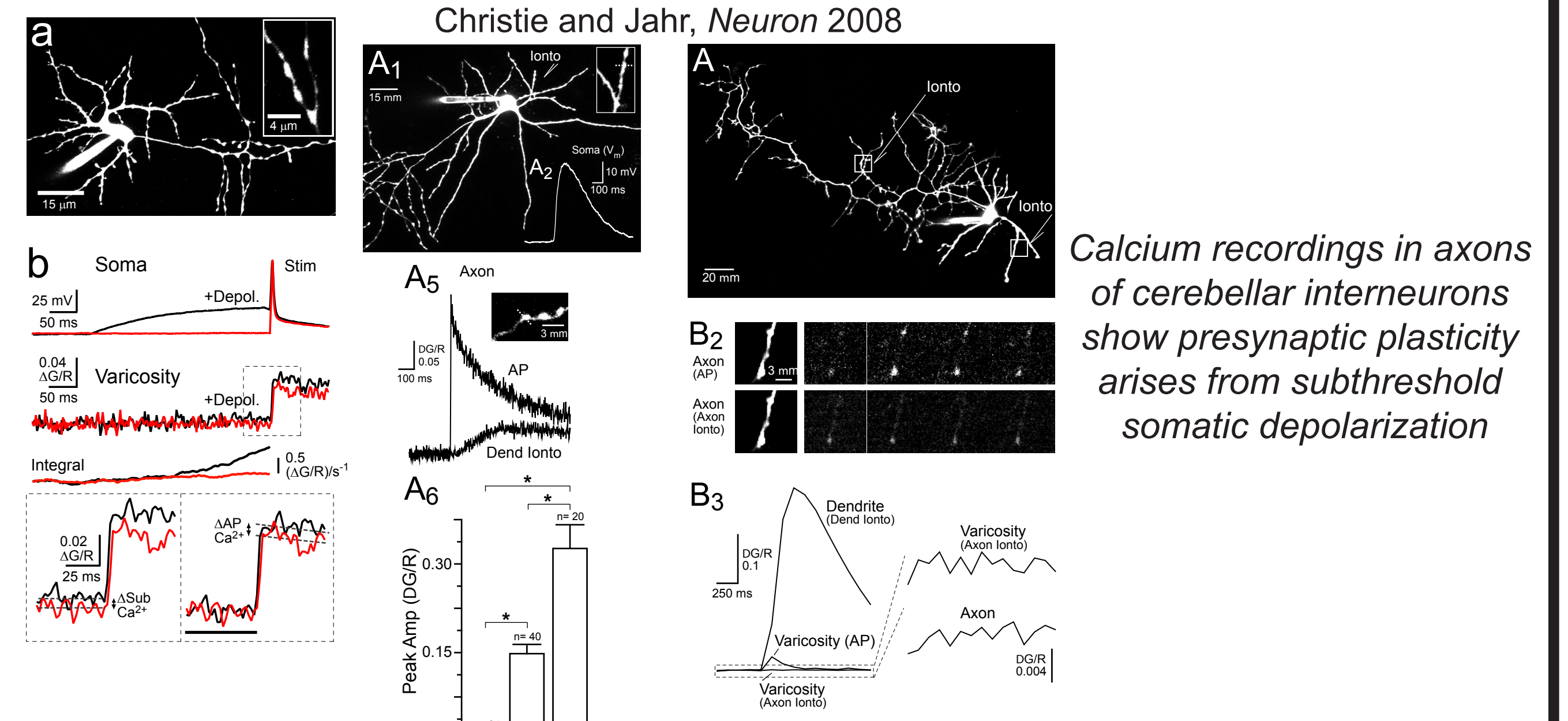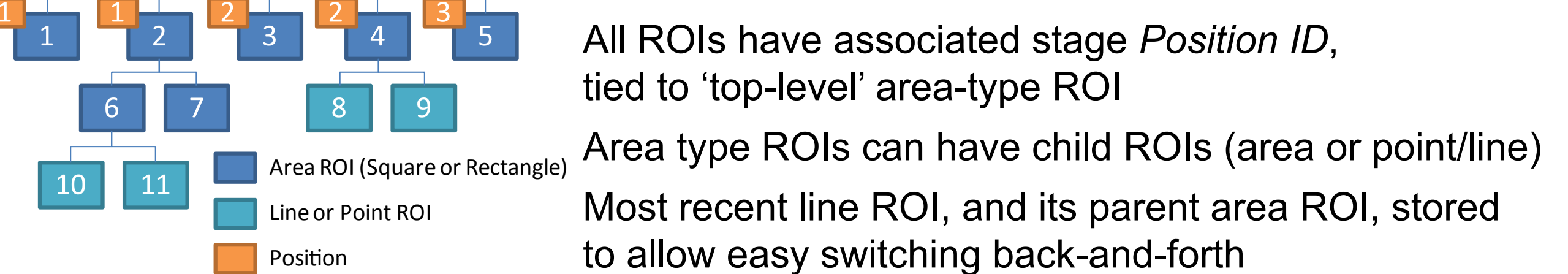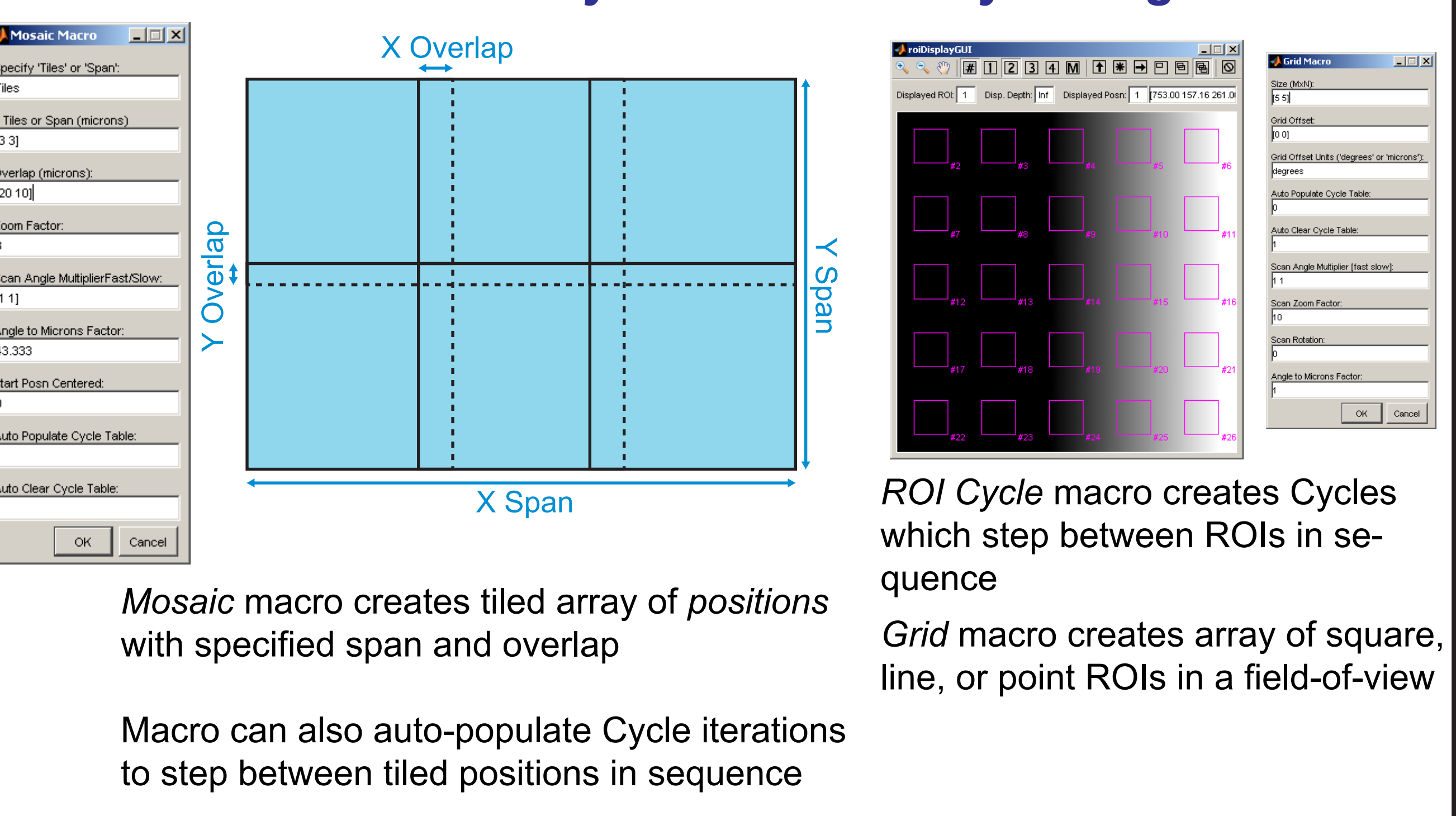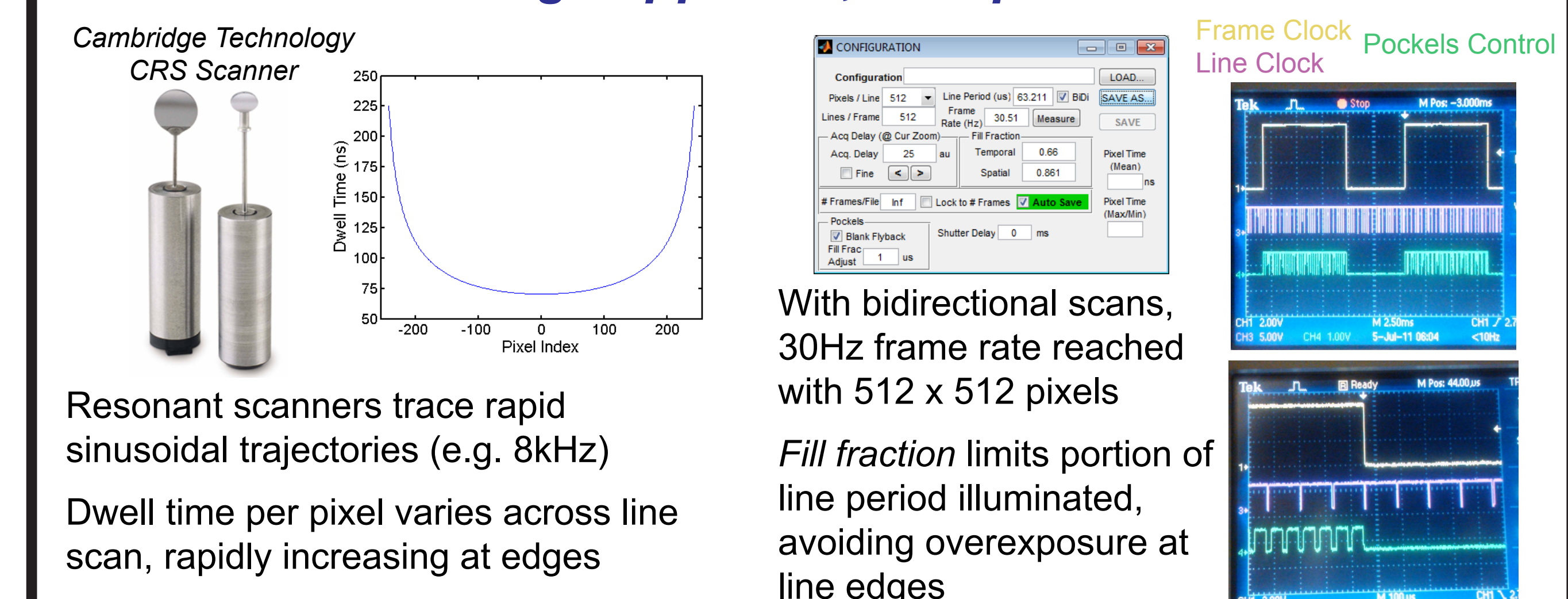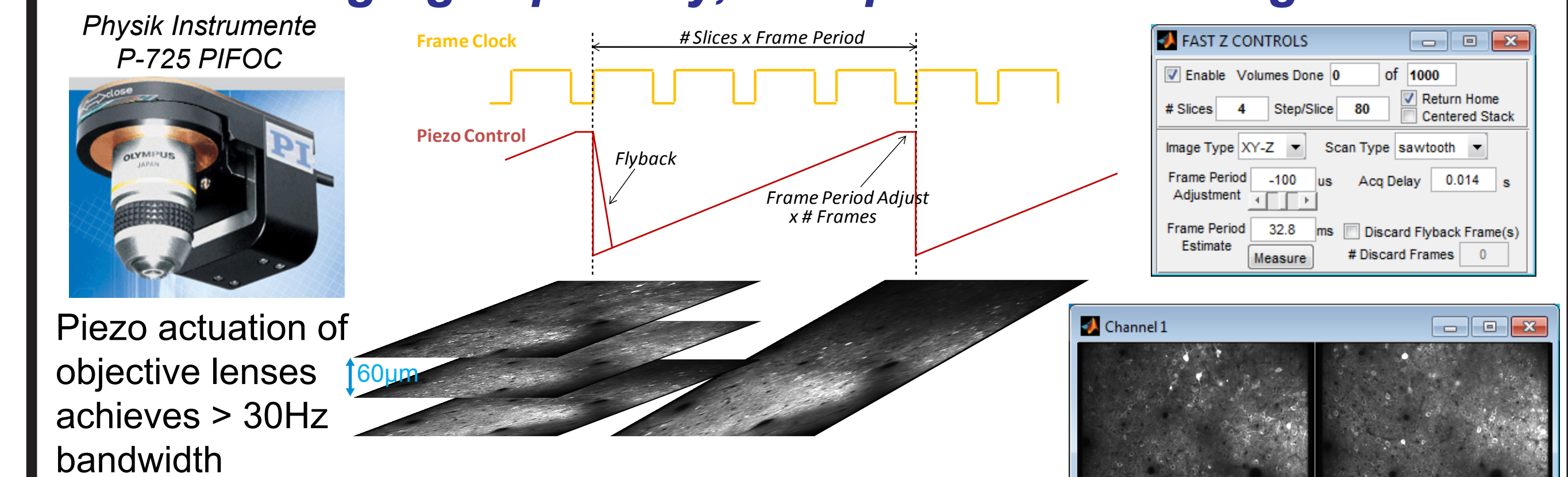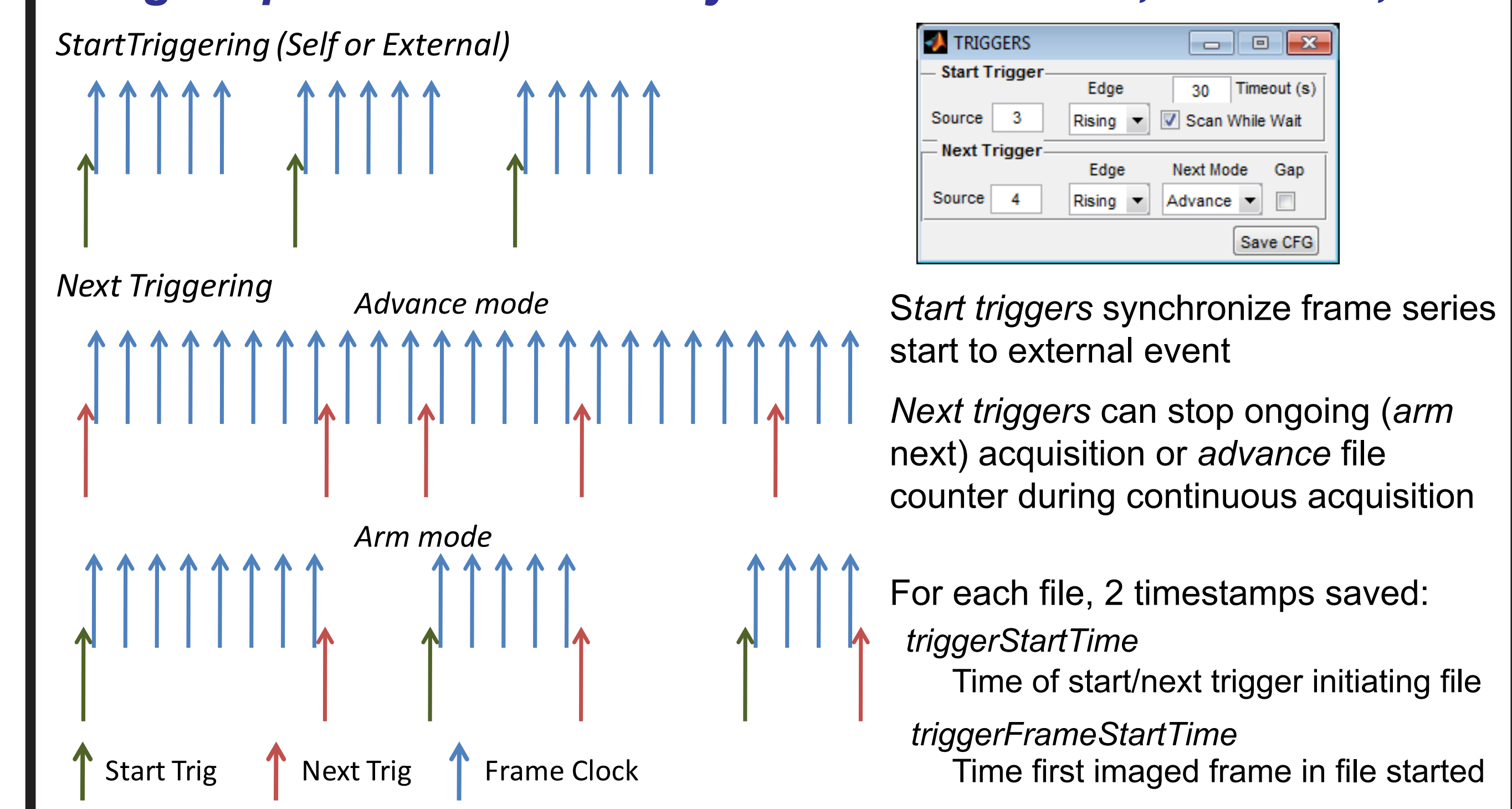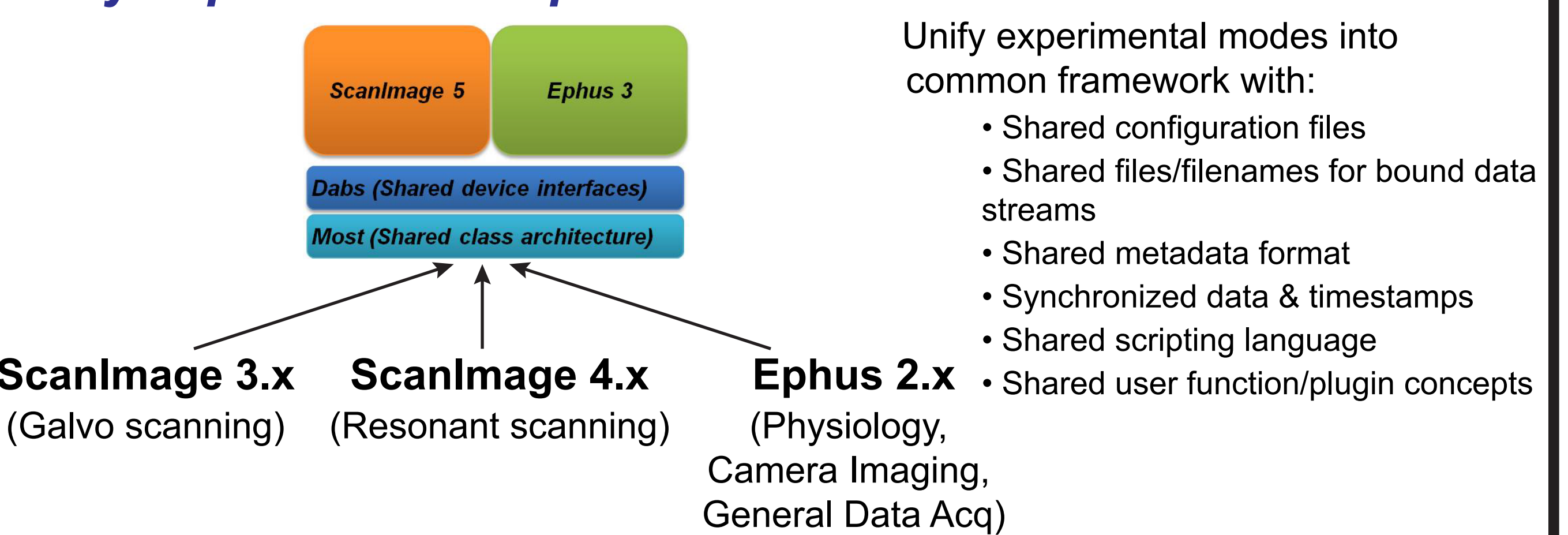E Interactive specification of stack start & end points

Position Controls (new)

● Store/recall stage *positions*, within session or across days
● Positions coordinated with ROIs in ScanImage 3.8

Distribution

ScanImage, and *Ephus,* are freely available at
http://scanimage.org
http://ephus.org

ScanImage & *Ephus*
● Over 300 registrations
● At or nearing 100 users (informal survey)

Actively using or trying ScanImage and/or Ephus?
Complete the annual survey!
http://surveymonkey.com/s/scanimage_ephus