ScanImage: extensible software for laser scanning microscopy

871.04
LLL65

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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. ScanImage is particularly optimized for continuous imaging in volumes of neural tissue, synchronized in time with behavioral data, sensory stimulation, and/or electrophysiology.

ScanImage Capabilities

Release 3.8.1 - Galvo Scanning (~1 kHz line rate)

Release 4.2 - Resonant Scanning (16 kHz line rate)
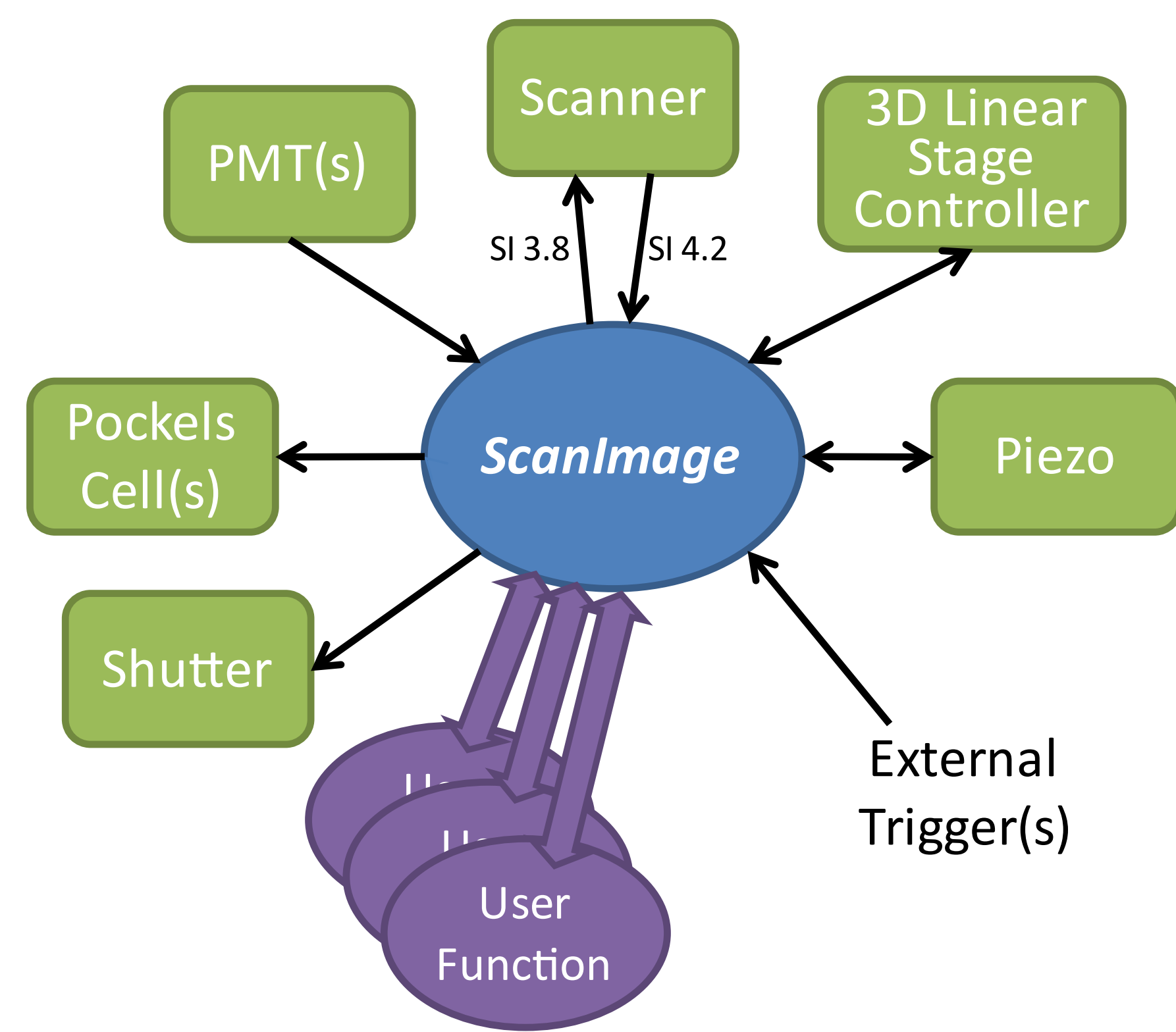
User Functions Concept

ScanImage 3.8.1 & 4.2 are extensible via user functions

ScanImage allows *binding* user functions (M or MEX files) to specific *events* during SI operation

Arguments can be supplied to bound user functions, allowing their operation to be modulated

User function bindings and arguments are stored as part of ScanImage *configuration files*

'Extension'

Binding same user function to multiple ScanImage events comprises a ScanImage *extension*

ScanImage automatically passes event name to user function, which uses *switch* statement

MATLAB *persistent* variables can be used to store state between events firing user function

Sample ScanImage Extensions (included with release)

postTrigPulse

periodTriggeredPulse

frameClockMultiplier

ScanImage extensions can be used to sync imaging to experiment

ScanImage includes NI DAQmx wrapper class in 'Dabs' package - NI counter/timer chan functionality supported

roiPlot

ScanImage extensions can be used for live analysis/visualization during imaging

Sample Applications

Encoding of object location during whisker locomotion
Nick Sofroniew...Karel Svoboda (HHMI/JFRC)

Neurons are differentially tuned to wall distance

2-Photon GCaMP6s imaging

L2/3 neurons in barrel cortex are tuned to wall distance in mouse navigating a virtual tactile maze

ScanImage 4.1
743.15

Modulation of sensory activity by wakefulness
H. Kato, M. Chu, J. Isaacson & T. Komiyama *Neuron* 2012 (UCSD)

Wakefulness causes mitral cell responses to be more sparse, more temporally dynamic, and more efficient at coding compared to anaesthesia

ScanImage 4

In Vivo *Targeted Dendritic Patching*
S. Smith, I. Smith, T. Branco, & M. Hausser *Nature* 2013 (UCL)

Dendritic patching targeted via two-photon imaging of filled neuron (AL 594)

Dendritic spikes enhance somatic visual orientation selectivity in awake rodents

ScanImage 3.8

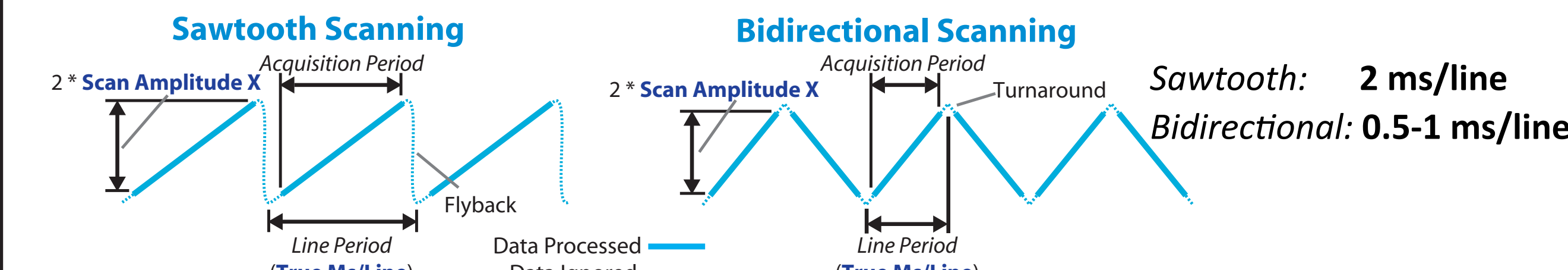ScanImage *supports a wide range of imaging applications in living neural tissue*

ScanImage 3.8.1

New Features
- MATLAB 2013b & DAQmx 9.8 compatibility
- PI E753 piezo controller support
- Luigs & Neumann stage support (by 2014)
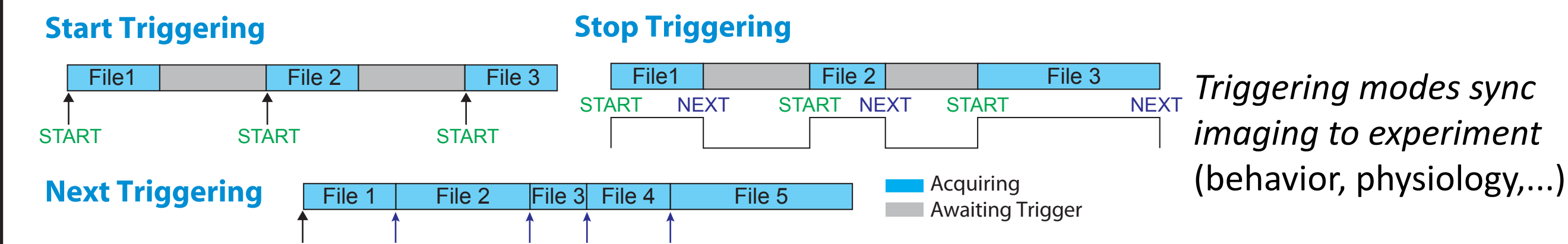- Npoint piezo support (by 2014)

Key Features & Concepts
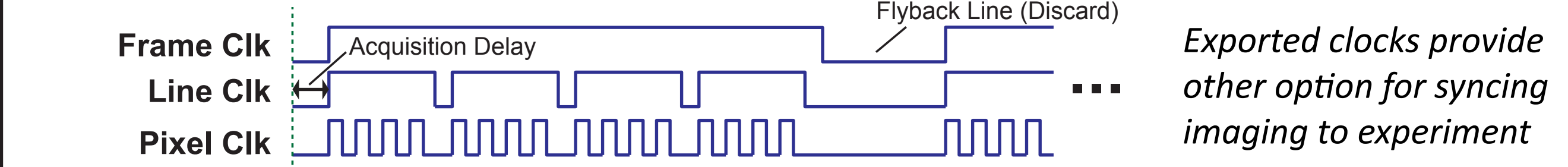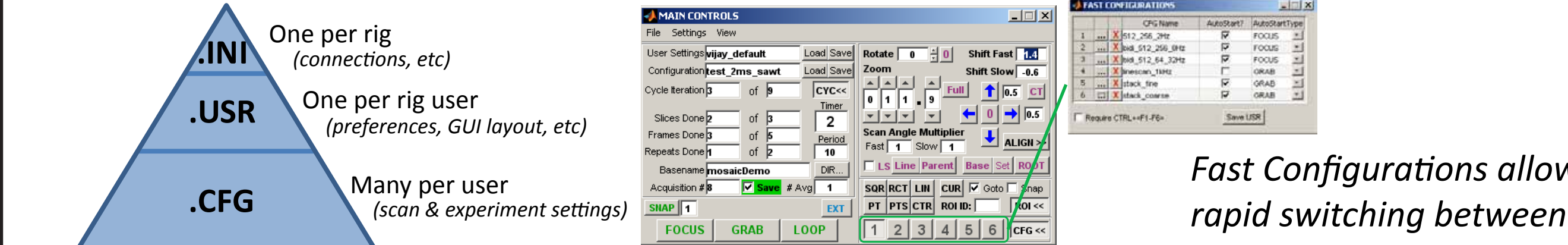
Sawtooth & Bidirectional Scanning

Sawtooth: **2 ms/line**
Bidirectional: **0.5-1 ms/line**

Hardware Flexibility

Generic stage controller interface allows flexibility to choose motor hardware

External Trigger Modes

Triggering modes sync imaging to experiment (behavior, physiology,...)

Exported Clocks

Exported clocks provide other option for syncing imaging to experiment

Hierarchical & Fast Configuration Files

.INI — One per rig (connections, etc)
.USR — One per rig user (preferences, GUI layout, etc)
.CFG — Many per user (scan & experiment settings)

Fast Configurations allow rapid switching between commonly used settings

ROI Imaging

ROI Scan Parameters
- Zoom
- Shift Fast/Slow
- Rotation
- Scan Angle Multiplier (SAM)

ROI Parameters allow rectangular, line, & point scans to be specified

Cycle Mode

Cycle Mode allows timed sequences of experimental settings, scan settings, and/or motor positions,

ScanImage 4.2

New Features
- MATLAB 2013b & DAQmx 9.8 compatibility
- Multi-ROI scanning capability (preliminary)
- Faster frame rates at low zoom (reduced galvo flyback times)
- X/Y Field Size calibration supported
- Pockels blanking during piezo flyback (Discard Flyback Frame)
- Allow lines/frame > pixels/line, e.g. for line scanning
- 'Stop Triggering' mode support
- Luigs & Neumann stage & Npoint piezo support (by 2014)

Volume Imaging

npoint nPFocus400

Piezo ramp & flyback synced to integer number of frame periods

Simon Peron et al (743.12)

total: 48,742 neurons across 5 animals

Multiple region-of-interest scanning (preliminary)

Combine resonant scanner with galvo scanner pair

LASER → RES SCANNER → GALVO X → GALVO Y → SCOPE

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

Pollen Grain Test

Imaging targeted to specific sub-ROIs allows higher total frame rate and/or higher resolution imaging

File Frame Format

| ROI 1 |
| ROI 2 |
| ROI 3 |
| ROI 4 |

Transit Time (~200 µs)

ROIs all share same # pixels/line and resonant scanner zoom factor. The # lines and aspect ratio can vary per ROI.

Scanimage 5

In 2014, ScanImage capabilities will be unified in 1 version*

ScanImage 5 - Galvo

ScanImage 5 - Resonant

Ephus 5
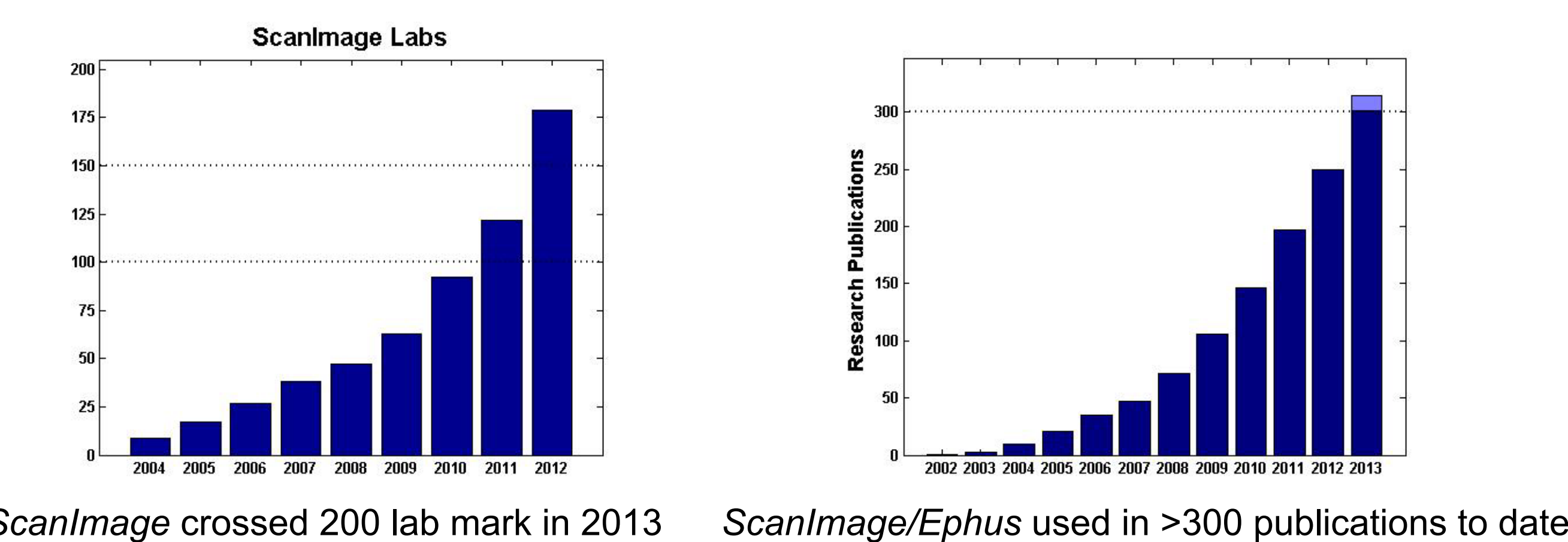
Shared Framework

Highlights
- Support for OEM resonant scanning hardware
- Completely scriptable interface
- Shared framework:
  - Shared file-naming & experiment control
  - Shared configuration & fast configuration files
  - Shared extension/plugin framework

Tell us what's important to YOUR lab & research!

* Pending funding

Distribution

ScanImage, and *Ephus,* are freely available at

http://scanimage.org

http://ephus.org

ScanImage Labs

Research Publications

ScanImage crossed 200 lab mark in 2013   *ScanImage/Ephus used in >300 publications to date*

- ScanImage/Ephus used in over 20 countries and over 25 US states
- About 95% of labs involved in neuroscience-related research