

# ScanImage: Flexible and modular software for laser scanning microscopy

816.11

Vijay Iyer<sup>1</sup>, Timothy O'Connor<sup>2</sup>, Daniel Huber<sup>1</sup>, Simon Peron<sup>1</sup>, Bart Borghuis<sup>1</sup>, Jayaram Chandrashekar<sup>1</sup>, Gordon M.G. Shepherd<sup>2,1</sup>, and Karel Svoboda<sup>1</sup>  
<sup>1</sup>HHMI/Janelia Farm Research Campus and <sup>2</sup>Dept. of Physiology, Northwestern University

## Overview

**ScanImage** is software to control laser scanning microscopes, particularly *two-photon microscopy* and *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 continuous, extended, and fast imaging in conjunction with behavioral data, sensory stimulation, and/or electrophysiology.

### RELEASE HIGHLIGHTS

#### Release 3.6/3.6.1 (December 2009/August 2010)

- Bidirectional scanning - scan rates of **0.5 ms/line**
- Enhanced scan configuration capabilities
- Support for 4 input channels, with adjustable ranges
- Improved data processing performance, allowing high frame rates

#### Release 3.7 (Beta Available NOW!)

- Automated power compensation during image stack collection
- Generation of frame/line/pixel clocks
- Enhanced triggering capabilities, including *next triggering*
- Enhanced *user function* capabilities enabling user customization
- DAQ Toolbox no longer required and faster DAQ performance

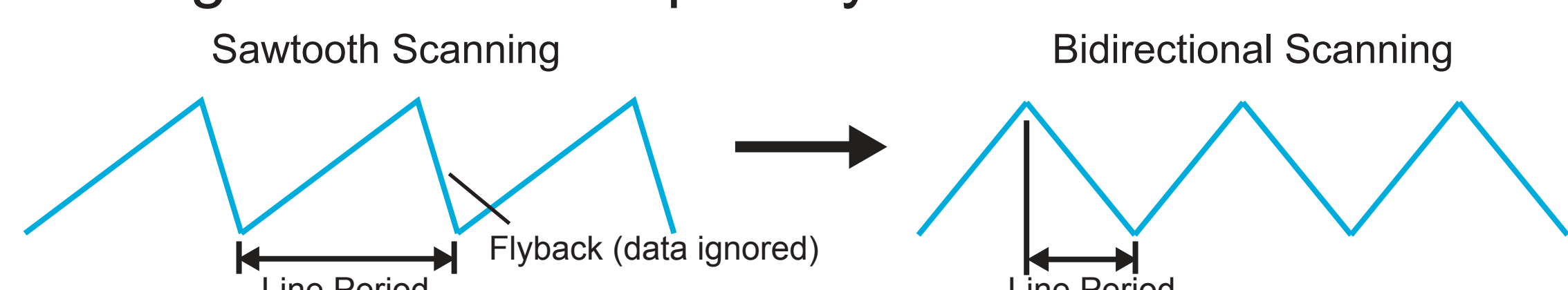
#### Release 4.0 & Beyond (In Progress)

- Faster raster scanning, using resonant scanning hardware
- Faster axial scanning, using piezoelectric actuators
- Multi-scanner support, e.g. for simultaneous photostimulation
- Random-access scanning

## ScanImage 3.6

ScanImage 3.6 was released in December 2009

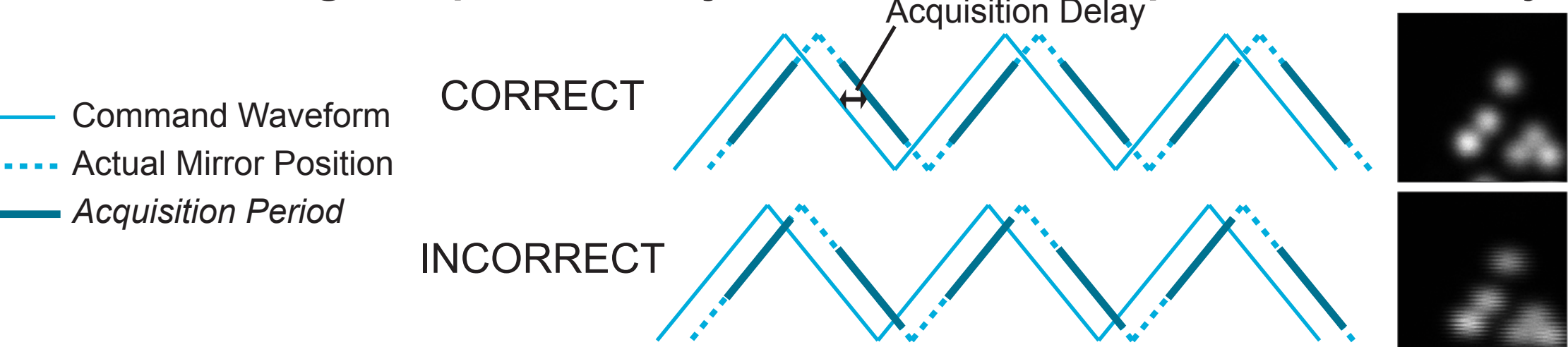
ScanImage 3.6 added capability for *bidirectional* scanning



Bidirectional (BID) 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.

ScanImage 3.6 enhanced scan configuration

**Acquisition Delay**  
Delay between command and scanner response

**Scan Delay**  
Delay from start of line period to *acquisition period*

**Fill Fraction**  
Fraction of line period occupied by *acquisition period*

**Acquisition Delay, Scan Delay, and Fill Fraction values can be stored for each Zoom factor**

CONFIGURATION dialog in r3.6

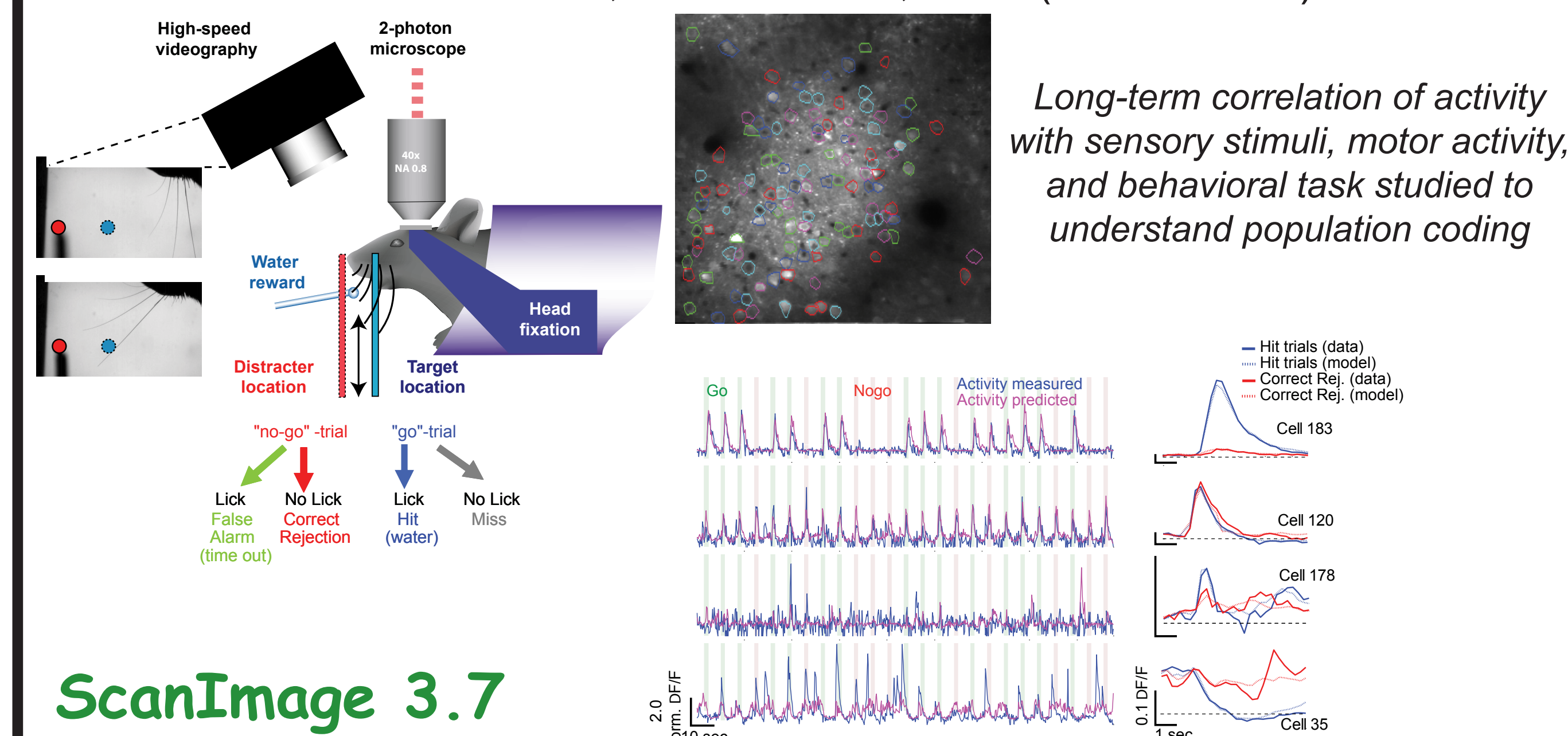
ScanImage 3.6.1 was released in August 2010

- Support for 2048 pixels/line
- Several small bugfixes

## Sample Applications

### Neuronal population coding in mouse motor cortex

Daniel Huber, Simon Peron, et al. (HHMI/JFRC)

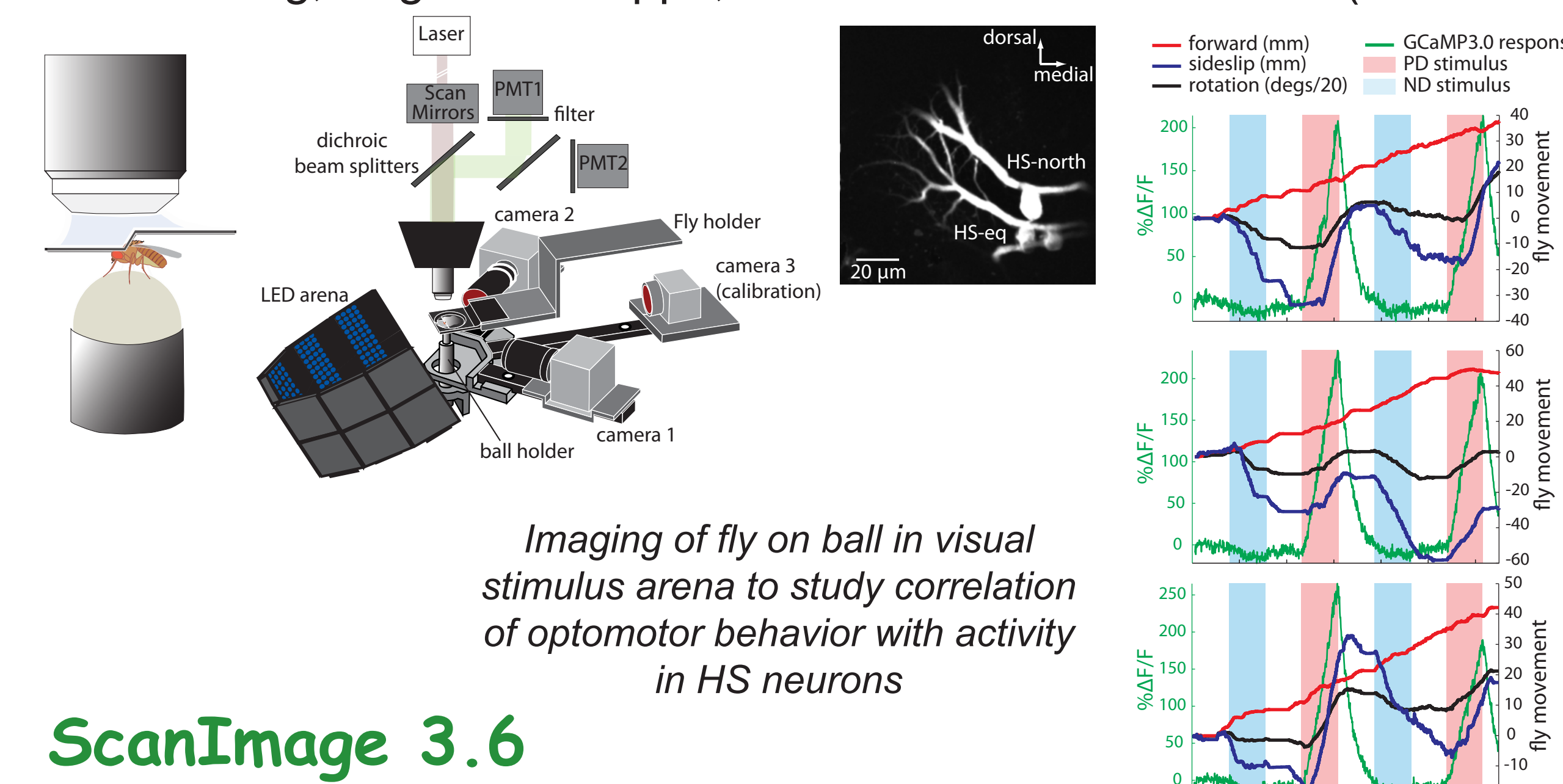


ScanImage 3.7

285.16

### Head-fixed Drosophila during optomotor walking behavior

Johannes Seelig, Eugenia Chiappe, et al. *Nature Methods* 2010 (HHMI/JFRC)

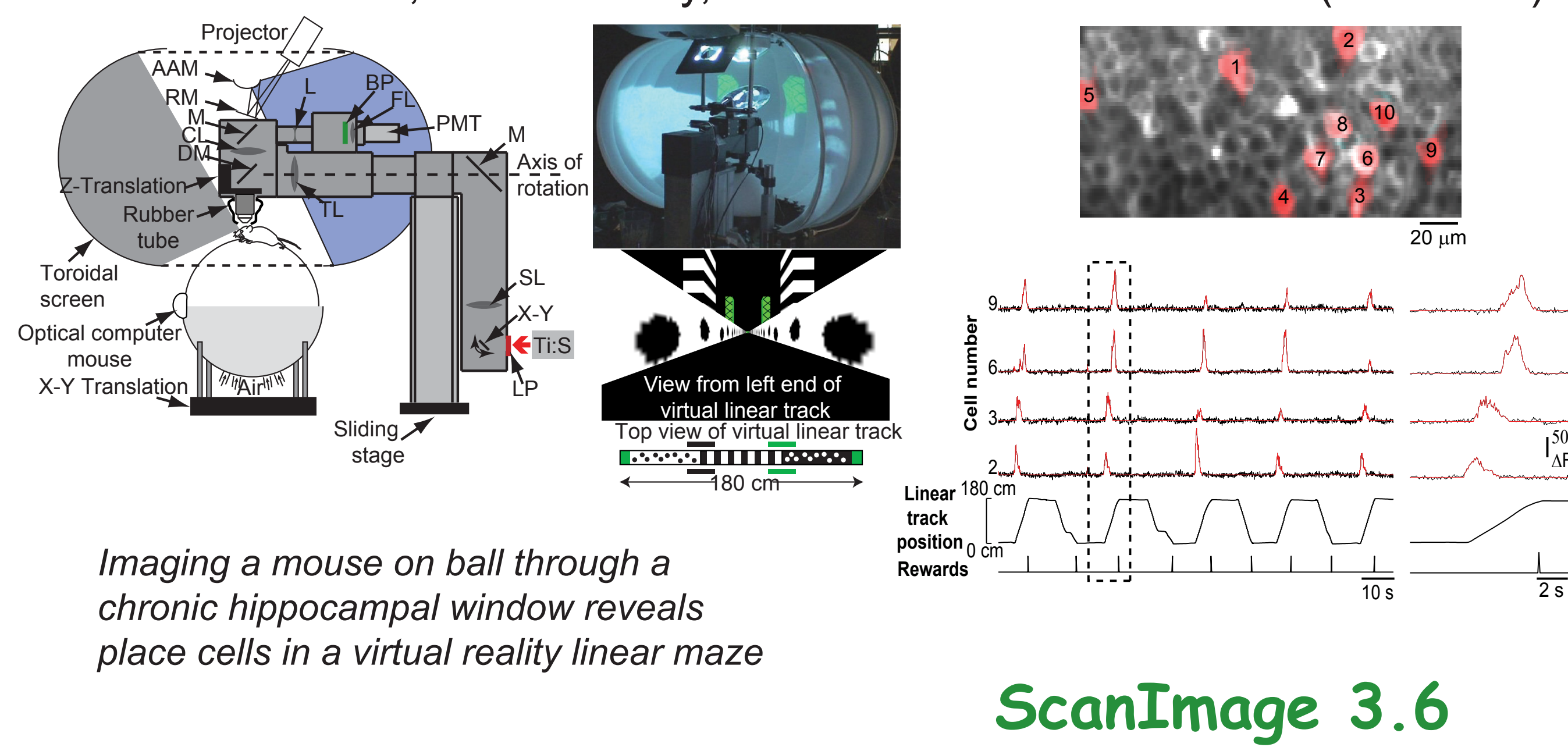


ScanImage 3.6

ScanImage supports advanced custom microscopy applications in behaving animals

### Hippocampal place cell activity during virtual navigation

Daniel Dombeck, Chris Harvey, et al. *Nature Neurosci.* 2010 (Princeton)



ScanImage 3.6

203.18

## ScanImage 3.7

### POWER vs DEPTH ADJUSTMENT

Power increases automatically with depth for image stacks

**P/z Adjust**  
Enable power adjustment during image stacks

**Lz**  
Exponential length constant for power adjustment (per Beam)

$$P(z) = P_0 e^{-(z-z_0)/Lz}$$

**Override Lz**  
Adjust and record power value at start and end of stack (specify during FOCUS)  
Lz is computed, overriding value in Power Controls

### FRAME/LINE/PIXEL CLOCK

External hardware (e.g. stimuli) can be synced to imaging

Frame Clk  
Line Clk  
Pixel Clk

Acquisition Delay  
Flyback Line (Discard)

Start Trigger

**Phase Shift**  
Delay clock edge relative to start of frame/line/pixel

**Pixel Fraction**  
Fraction of pixel period that Pixel Clock maintains level after clock edge

### ADVANCED TRIGGERING

Multiple external trigger sources and triggering modes

**Start Trigger** Initiates image acquisition

**Next Trigger** Stops acquisition or advances data file during ongoing acquisition

**Typical Use Cases**

Image files aligned with behavior epochs

External Trigger Enable

### USER FUNCTIONS

User functions executed during ScanImage events

User function arguments allow 'plugins' to be created with a single function

User functions are Matlab functions (M/MEX) with signature:  
`function myFunc(eventName,eventData,optArg1,optArg2,...)`

- ScanImage passes *eventName* & *eventData* to user function
- Optional **Arguments** allow configurable modulation of user function
- Multiple user functions can be bound to each event (**FcnIndex**)

### FAST CONFIGURATIONS

Fast Configurations loaded/started with single button

Six Configuration (CFG) files can be assigned as *Fast Configurations*

- *Fast Configurations* selected either on **Main Controls** or **F1-F6** keys
- **AutoStart?** specifies acquisition (of specified type) is started immediately after loading CFG file

### DAQmx ADAPTOR CLASS

DAQ Toolbox replaced by fast comprehensive adaptor

National Instruments DAQmx package is first of growing library of device adaptors implemented as Matlab classes

	DAQ Toolbox	DAQmx Package
Read Time	Slower	Faster
Streaming digital I/O	✗	✓
Counter/Timer Channels	✗	✓

DAQmx package has several advantages over DAQ toolbox

DAQmx package enables *frame/line/pixel clock* and *next triggering* in ScanImage 3.7

DAQmx concepts (e.g. **Task**) directly mapped to Matlab objects consisting of *properties* & *methods*

```
>> import DABS.Devices.NI.DAQmx.*
>> hTask = Task('Sample Task');
>> hTask.createAnalogChan('Dev1_0');
>> hTask.sampTimingType = 'DAQmx_Val_SampClk';
>> hTask.start();
```

## ScanImage 4.0

Support for additional, faster hardware underway

Resonant scanners allow 8x the line rate of servo-based scanners

- Faster frame rates (preserving lines/frame)
- Reduced photobleaching & photodamage
- Better motion detection (enabling correction)

Device adaptor to Thorlabs resonant scanner hardware, and prototype application, have been developed

Multi-threaded device adaptor allows high data rate and device event signaling to Matlab

Support for piezoelectric actuated axial motion also in progress

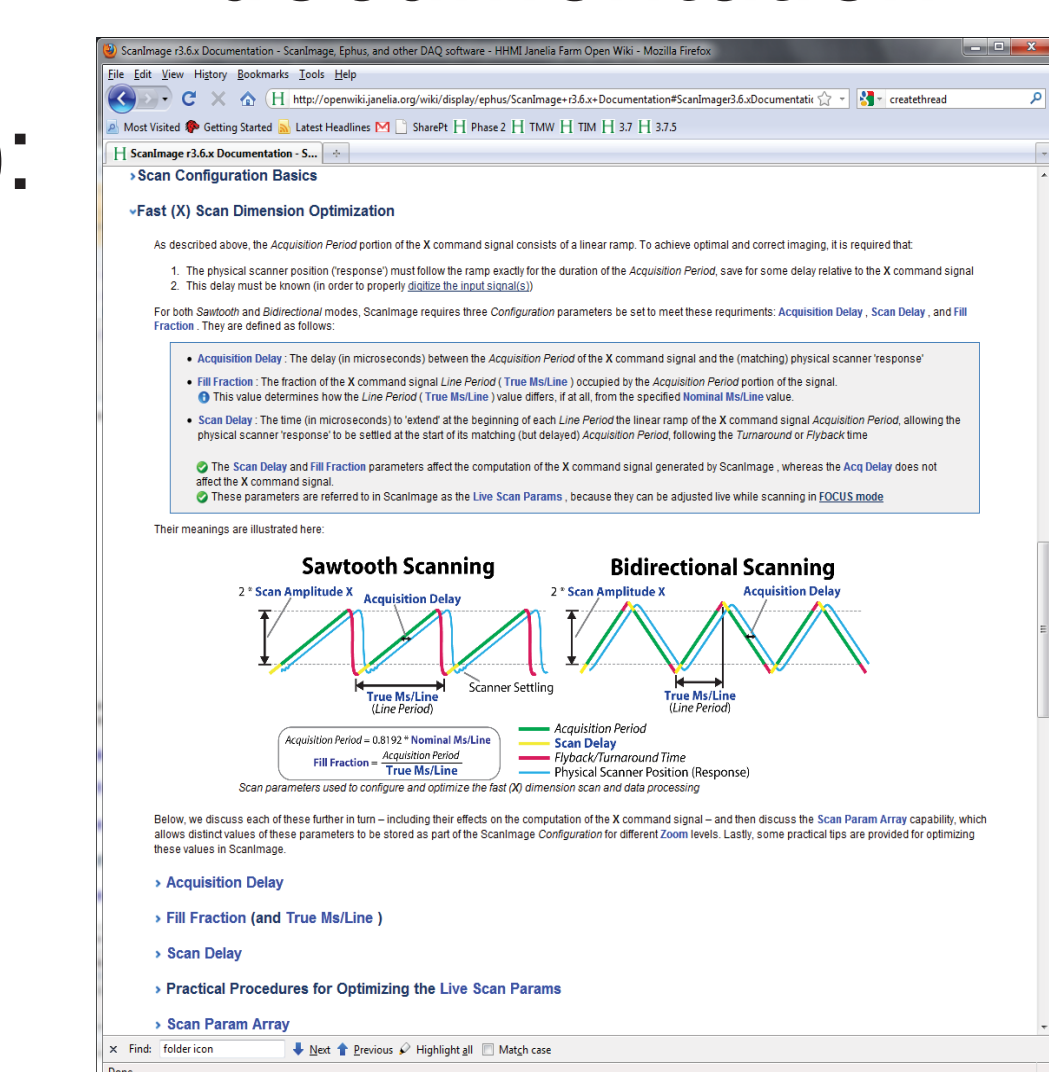
## Distribution

ScanImage is freely available at [www.scanimage.org](http://www.scanimage.org)

Extensive wiki style documentation

ScanImage & Ephus (816.10):

- Over 200 registrations (20 countries, 25 states)
- Over 75 confirmed users (>60 ScanImage)



**ACKNOWLEDGMENTS** We thank Tsai-wen Chen, Leopoldo Petreanu, Haining Zhong, and Ning-long Xu for testing beta versions and offering valuable feedback. Support from HHMI and NIH R01EB001464.