Design of a Coordinated System for Real-time 3-D Image Construction via Confocal Microscopy

Nathan Shepard

Master's Thesis Defense

September 17, 2007

Project Goals

- Coordinating imaging hardware to achieve:
  - Speed
  - Reproducibility
  - Automation of complex imaging patterns/overlays
  - Complete control from one screen
  - Automatic documentation
  - Hardware-specific, yet easy to modify

Outline

- Introduction
- History of Work
- Final Imaging Workflow
- Software Demonstration
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- Questions

Silva Research Group

- Purpose
  - Understanding CNS information processing
  - Quantitatively measuring activity of neural networks

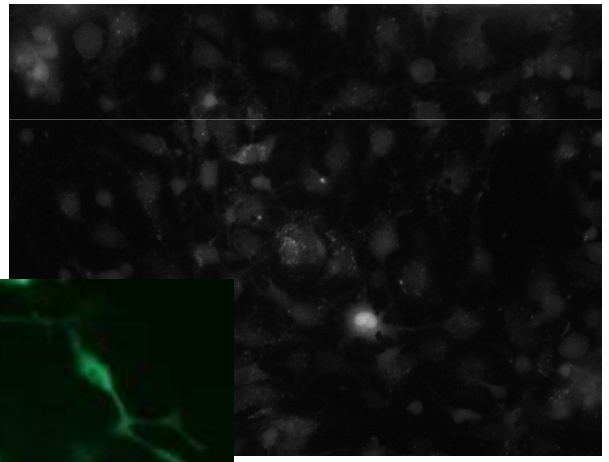
Functional clinical regeneration of CNS

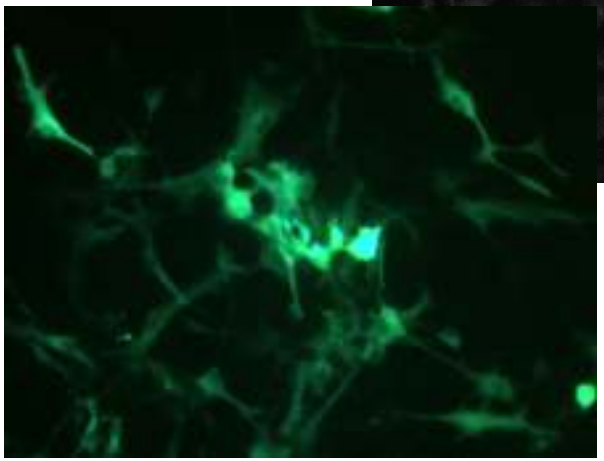
- Process
  - Using neural retina as CNS model
  - Confocal microscopy of neuronal and glial networks

Current Microscopy Projects

- Calcium-wave Imaging
  - Primary known mechanism for astrocyte activation
  - Process: Proportional or Ratiometric

- Confocal microscopy used to measure:
  - $[Ca^{2+}]$ Fluor-4 AM (white)
  - $[Ca^{2+}]$ Premo Cameleon (unbound = cyan) (bound = yellow)



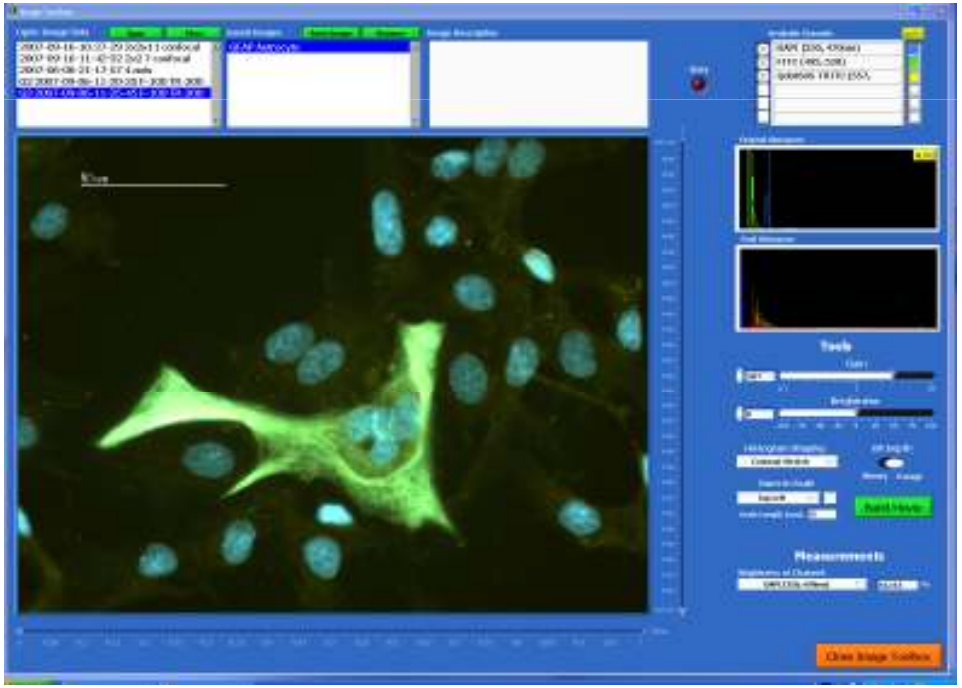


Movies courtesy of Diana Yu, Silva Research Group

Current Microscopy Projects

- Up-regulation of GFAP and Vimentin
  - Towards treatment for gliosis
  - Testing ability of anti-gliotic X to reduce IF proteins using ICC

- Confocal microscopy used to measure:
  - GFAP using FITC fluorophore (green)
  - Vimentin using TRITC fluorophore (yellow)
  - GFAP using Q-dots (orange, not shown)
  - DNA using DAPI (blue)

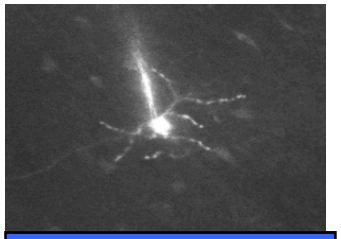


Confocal Microscopy Hardware

Hamamatsu Camera

Firewire

Scope PC
LabVIEW



1GB Ethernet

4-core
Linux

Olympus IX-81
Scope

RS232

Scion Stage
Controller

Lambda DG-4

Sutter 10-3

ScopePC/LabVIEW	4-core PC
* Controls devices * Records Images * Sends images and scope data (position, magnification) info to 4-corePC in real-time	Receives images and image position data Reconstructs 3D structure Displays 4D structure (3D structure plus time) of network and Ca waves. Allows rotation and visualization, with network structure overlay

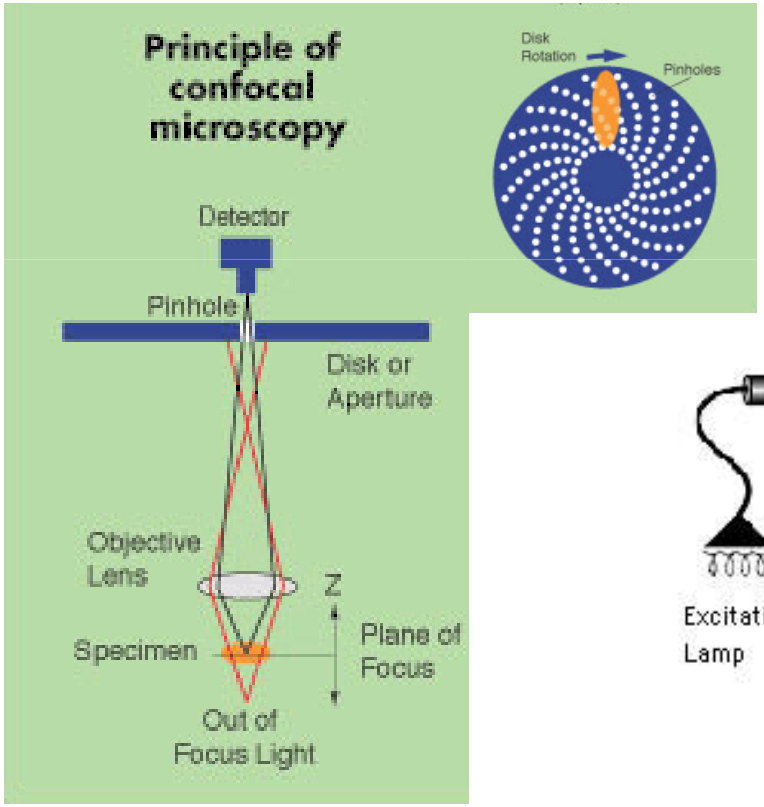
Image from www.swindale.ecc.ubc.ca/gallery.html

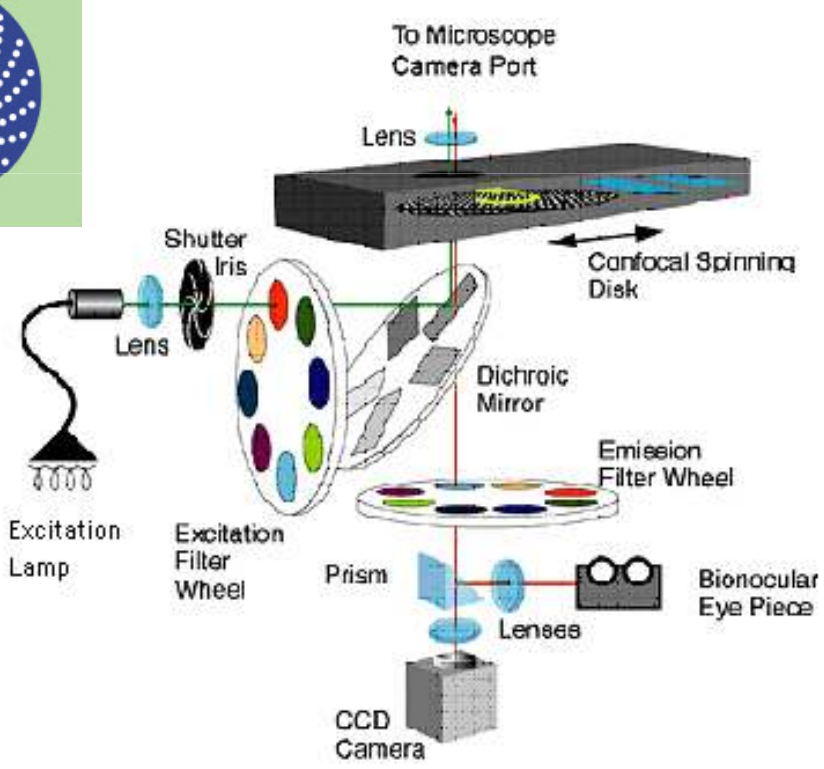
Confocal Microscopy Hardware

- Silva Lab imaging room

Confocal Microscopy

- Remove out-of-focus light to produce a high Z-resolution image





Images from BioVision Technologies www.biovis.com

Outline

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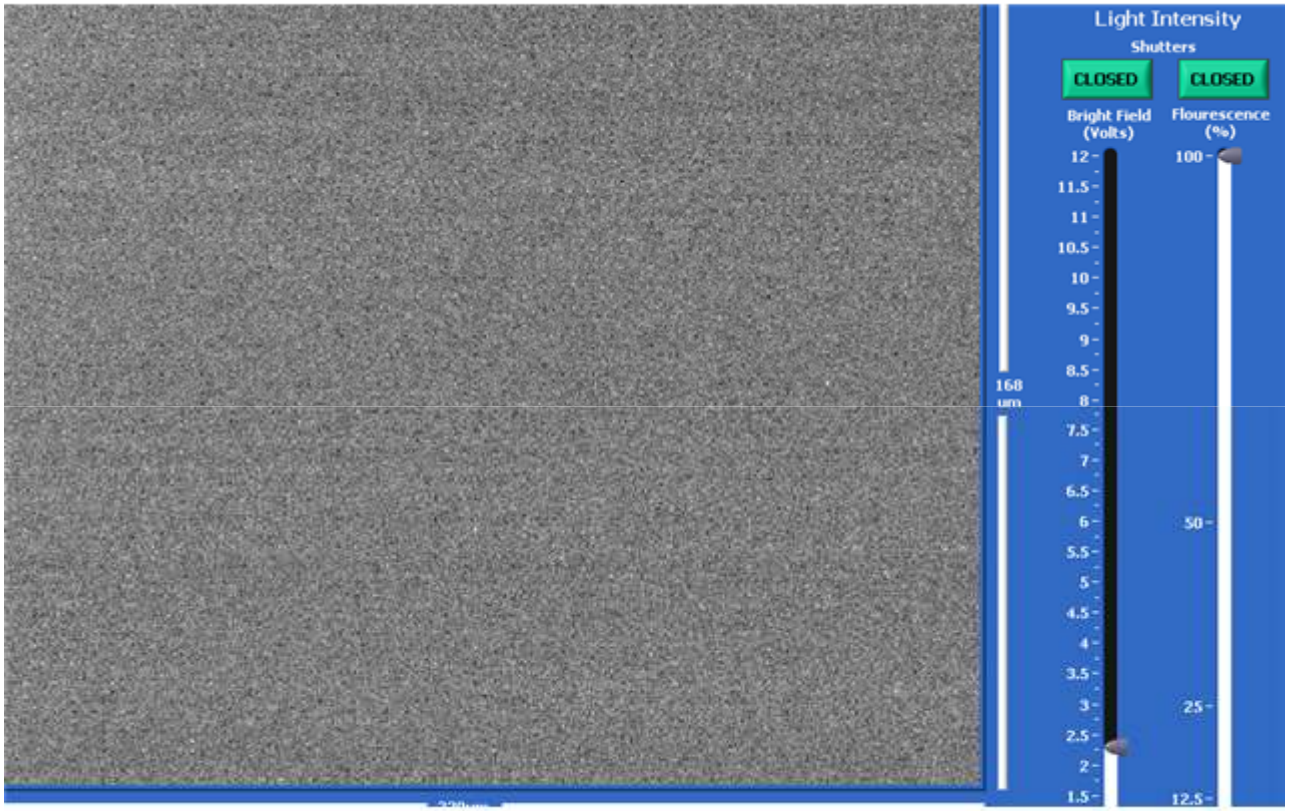
Why LabVIEW?

- Criteria for development environment
  - High volume signal processing
  - Time critical decisions at ~ 150Hz
  - Event-driven architecture
  - User-friendly controls
  - Native, optimized image processing
  - Minimum memory/processing footprint
  - Robust development environment
- Previous experience with LabVIEW
  - Realtime high-freq control of synchronous alternators
  - Realtime VEP recording and analysis
  - Realtime pupil-tracking via video input

Why LabVIEW?



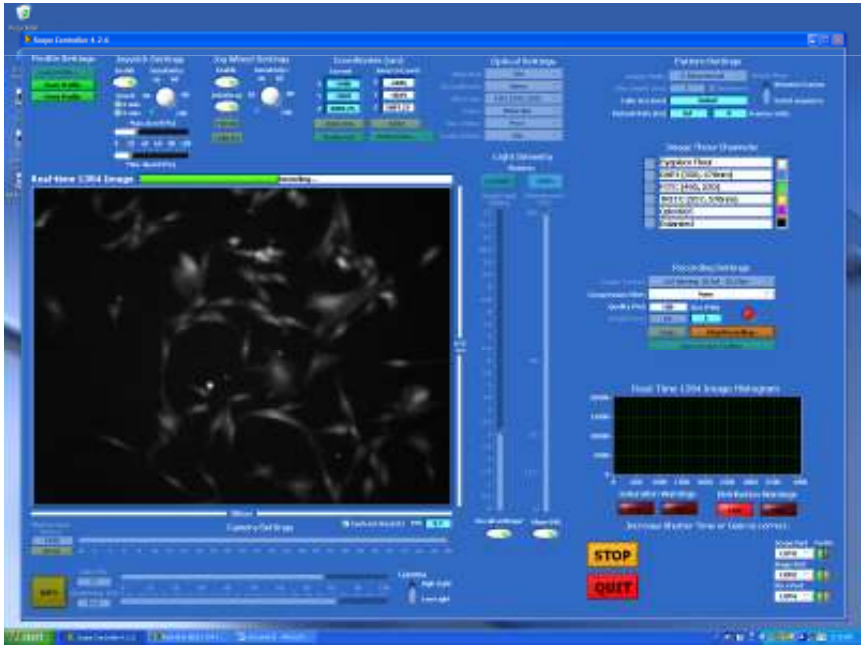
LabVIEW block diagram programming environment

Hardware Controls

- Optical controls
  - Illumination/Excitation
    - Bright Field (34), shutter (2)
    - Fluorescence (4), shutter (2)
  - Objective (6)
  - Filters
    - Confocal Disk (2)
    - DSU Cube (6)
    - Sutter Wheel (6)
    - IX Condenser (6)
    - Neutral Density (6)
    - Light prism (2)
- Subject position (X, Y, Z axes)
  - Maximum velocity
  - Acceleration profile
- Hardware settings
  - Joystick
    - Sensitivity (10)
    - Axis polarity (4)
  - Jog wheel sensitivity (10)
  - Button functions (28)
- Digital Imaging
  - Image Format (7)
    - Standard (3)
    - Format 7 (4)
  - Camera Settings
    - Gain, brightness, etc
  - Recording (4)

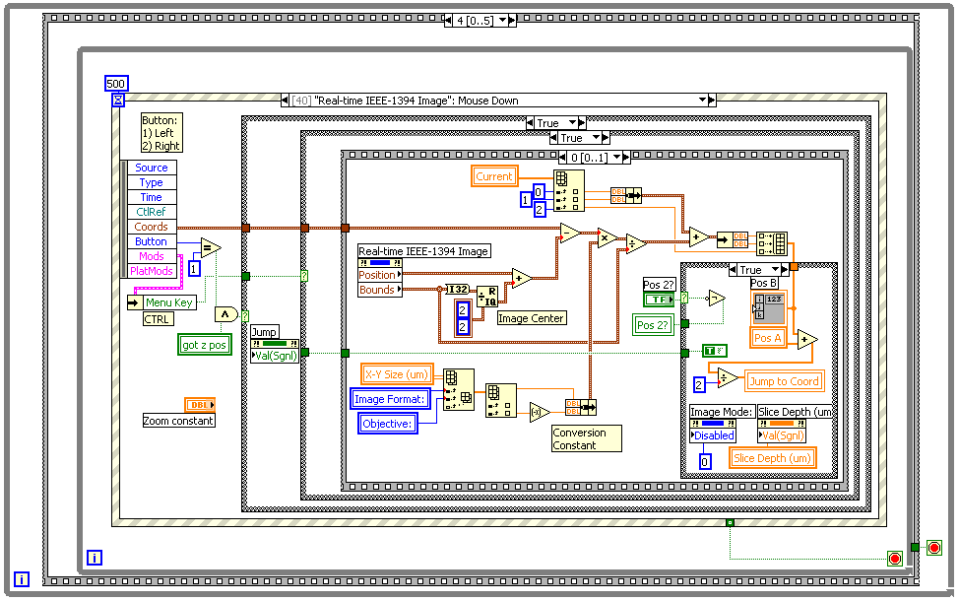
Imaging Features

- Profiles
  - Save exact settings for particular experiment
  - Recall focus, sensitivity, and exposure settings
- Mouse position control
  - Bookmark locations
  - Click-to-center
  - Scroll-to-focus
  - CTRL-click to measure
- Maintenance
  - Hardware reporting
  - Auto-calibration
  - Soft reset



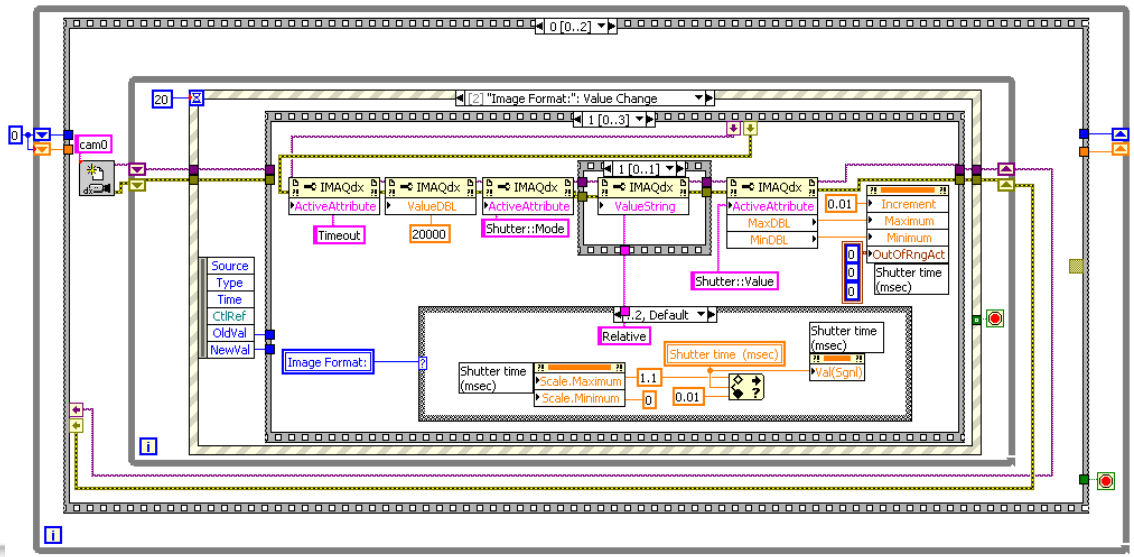
Imaging Features

- Real-time Imaging
  - Duplicate, true full-screen image
  - Histogram with analysis and advice
  - Camera - Auto-gain, brightness, gamma
  - Per channel settings
  - Contrast stretch
  - Record to disk
- Image Toolbox
  - Color mapping
  - Burnt-in scale
  - Histogram tools
  - Gain/brightness

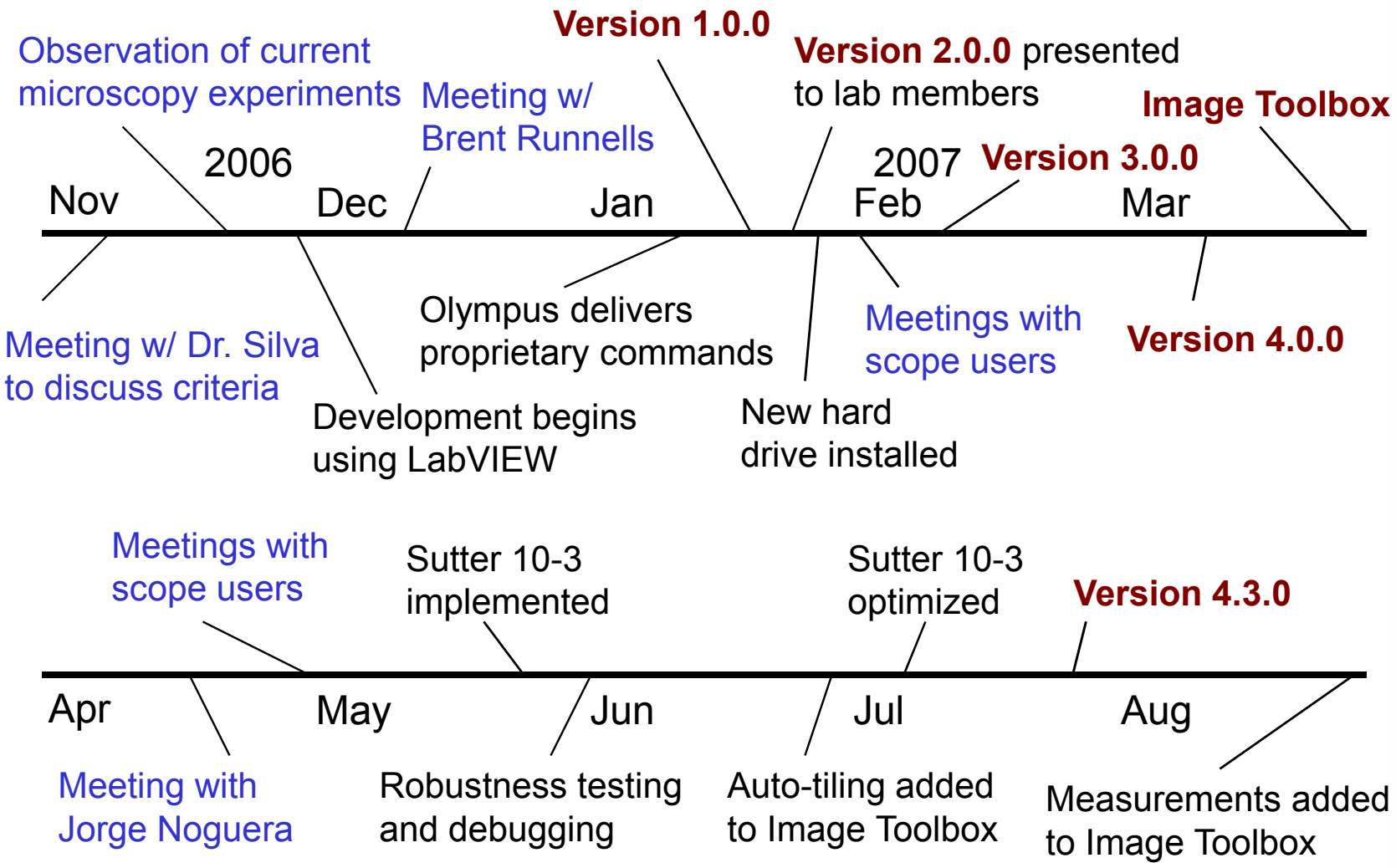


Implementation Structure

- Camera Module
- Communication Module
  - Send/receive commands/status
- User Interface Module
  - Manage primary user input
  - Manage secondary user input
- Recording Module
  - Pattern generation
  - Memory allocation
  - Real-time file save
  - Real-time documentation

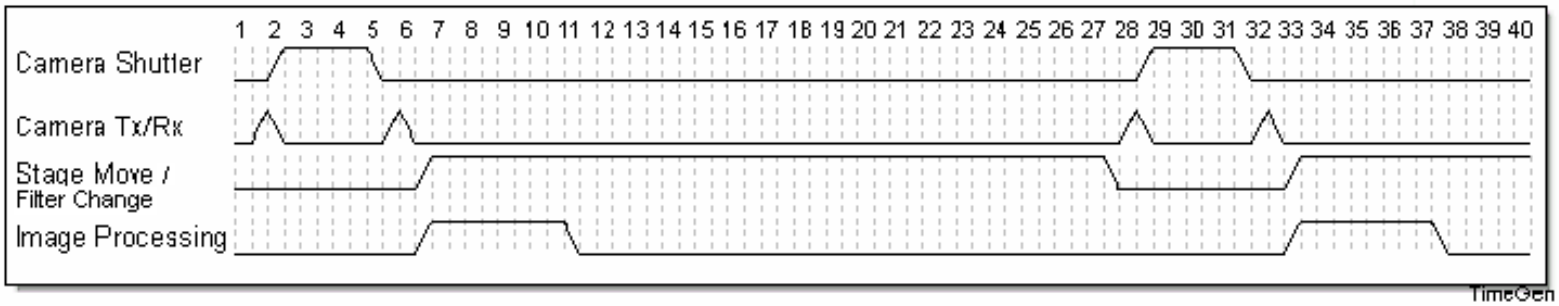


Development Timeline



Development Challenges

- Hamamatsu Camera
  - Driver conflict with ImagePro
  - Non-compliancy with many IEEE-1394 standards
- Memory leaks, large volume of data
- Image file storage/file name format
- Timing for Sutter 10-3 emission filter switch



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Types of Imaging

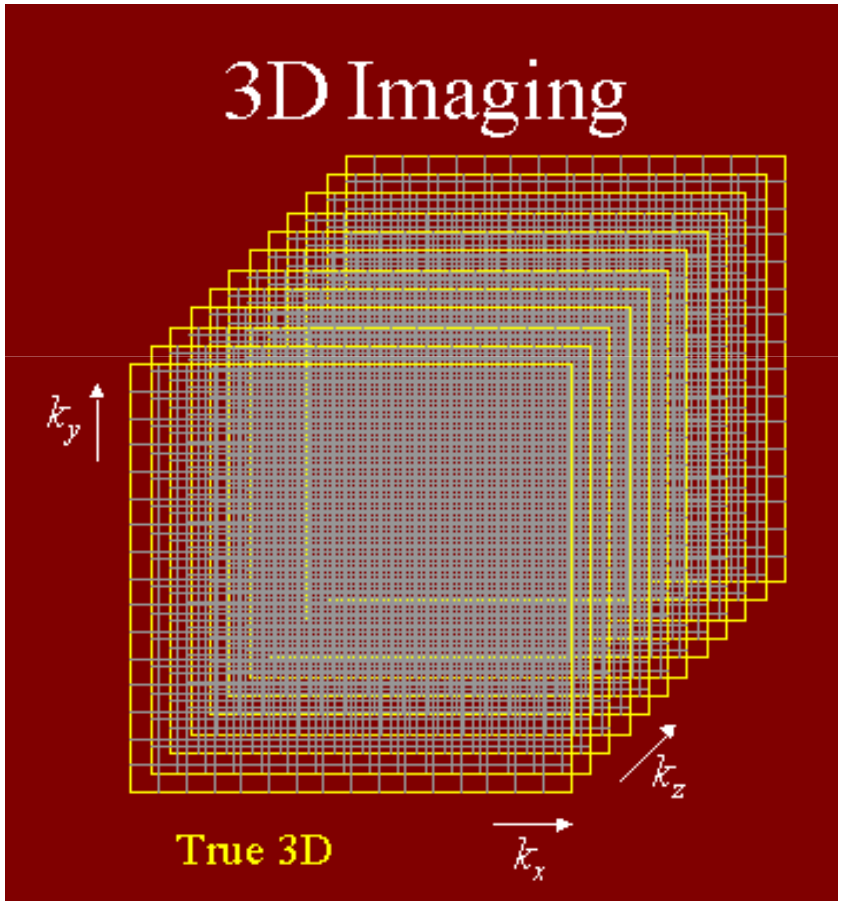
	Single Timestamp	Multiple Timestamps
Single imaging location	‘Still Snap’ Record one image on each channel	‘Still Movie’ Repeat recording one image per channel with timestamps for a pre-defined period of time
Multiple imaging locations	‘3-D Snap’ Record one image on each channel, per image location	‘3-D Movie’ Repeat ‘3-D Snap’ recording, with timestamps, for a pre-defined period of time

‘Still’ Imaging Workflow

- Set imaging location, focus
- For each channel to be imaged
  - Set exposure settings
  - Enable recording in channel list
- Choose recording type
  - ‘Snap’ - take one image of each channel
  - ‘Movie’ - choose movie length (sec)

‘3-D’ Imaging Workflow

- User sets two (X,Y,Z) coordinates
  - Top left
  - Bottom right
- User specifies Z-axis resolution (slice depth)
  - Balance resolution with sampling rate
- Image space is automatically compiled and stage/filter motion optimized



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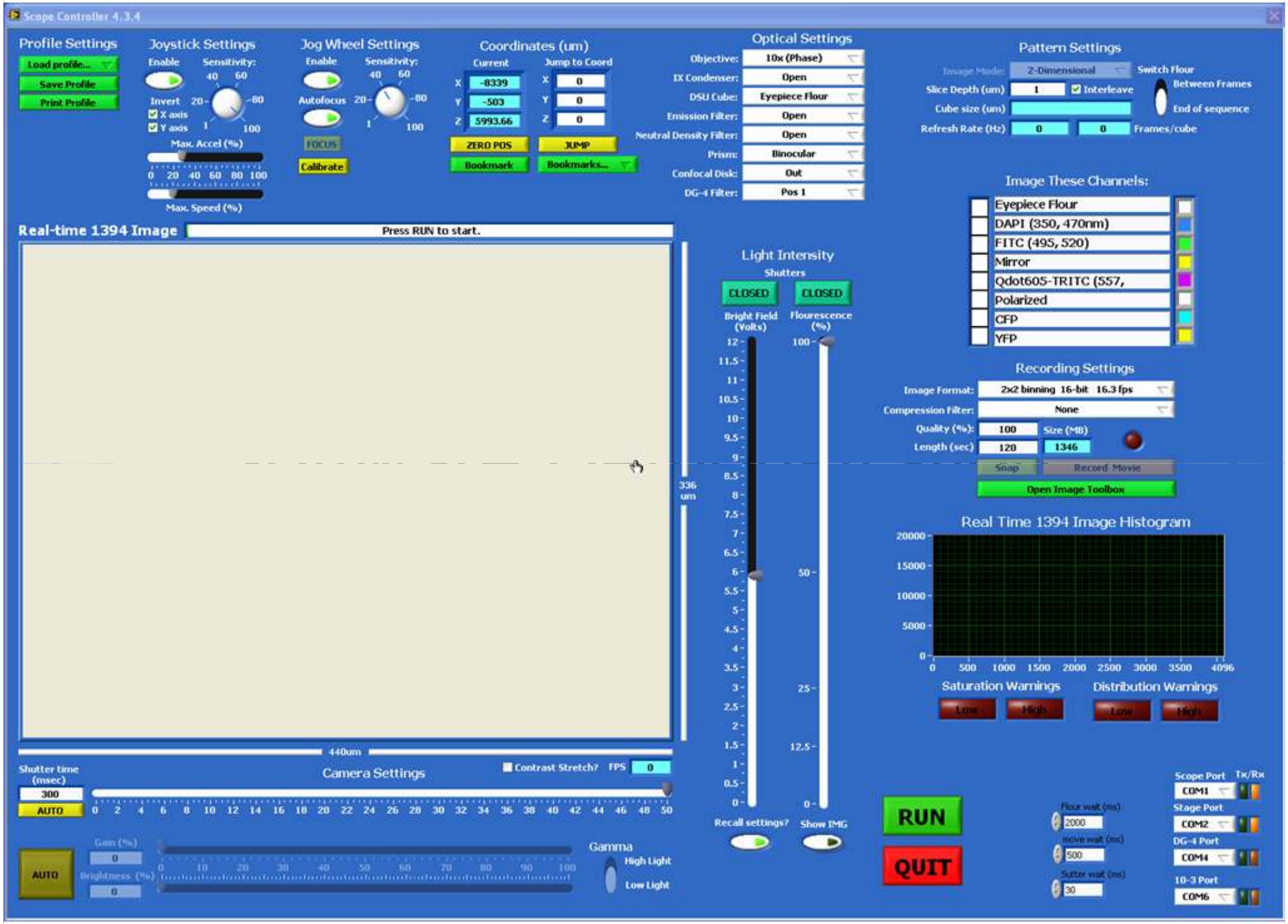
Software Demonstration


Scope Controller 4.3.4
Profile Settings
Load profile...
Save Profile
Print Profile
Joystick Settings
Enable
Sensitivity:
Invert
X axis
Y axis
Max. Accel (%)
Max. Speed (%)
Jog Wheel Settings
Enable
Autofocus
FOCUS
Calibrate
Sensitivity:
Coordinates (um)
Current
Jump to Coord
ZERO POS
JUMP
Bookmark
Bookmarks...
Optical Settings
Objective: 10x (Phase)
IX Condenser: Open
DSU Cube: Eyepiece Flour
Emission Filter: Open
Neutral Density Filter: Open
Prism: Binocular
Confocal Disk: Out
DG-4 Filter: Pos 1
Pattern Settings
Image Mode: 2-Dimensional
Switch Flour
Between Frames
End of sequence
Slice Depth (um)
Interleave
Cube size (um)
Refresh Rate (Hz)
Frames/cube
Image These Channels:
Eyepiece Flour
DAPI (350, 470nm)
FITC (495, 520)
Mirror
Qdot605-TRITC (557,
Polarized
CFP
YFP
Real-time 1394 Image
Press RUN to start.
336 um
440um
Light Intensity
Shutters
CLOSED
CLOSED
Bright Field (Volts)
Flourescence (%)
Recall settings?
Show IMG
Recording Settings
Image Format: 2x2 binning 16-bit 16.3 fps
Compression Filter: None
Quality (%): 100
Size (MB)
Length (sec): 120
1346
Snap
Record Movie
Open Image Toolbox
Real Time 1394 Image Histogram
Saturation Warnings
Low
High
Distribution Warnings
Low
High
Shutter time (msec)
300
AUTO
Camera Settings
Contrast Stretch?
FPS
0
Gain (%)
Brightness (%)
AUTO
Gamma
High Light
Low Light
RUN
QUIT
Flour wait (ms)
2000
move wait (ms)
500
Sutter wait (ms)
30
Scope Port
Tx/Rx
COM1
Stage Port
COM2
DG-4 Port
COM4
10-3 Port
COM6

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LabVIEW

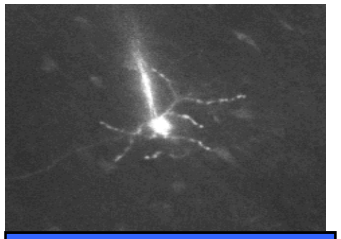

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Image from www.swindale.ecc.ubc.ca/gallery.html

Real-time 3-D Rendering

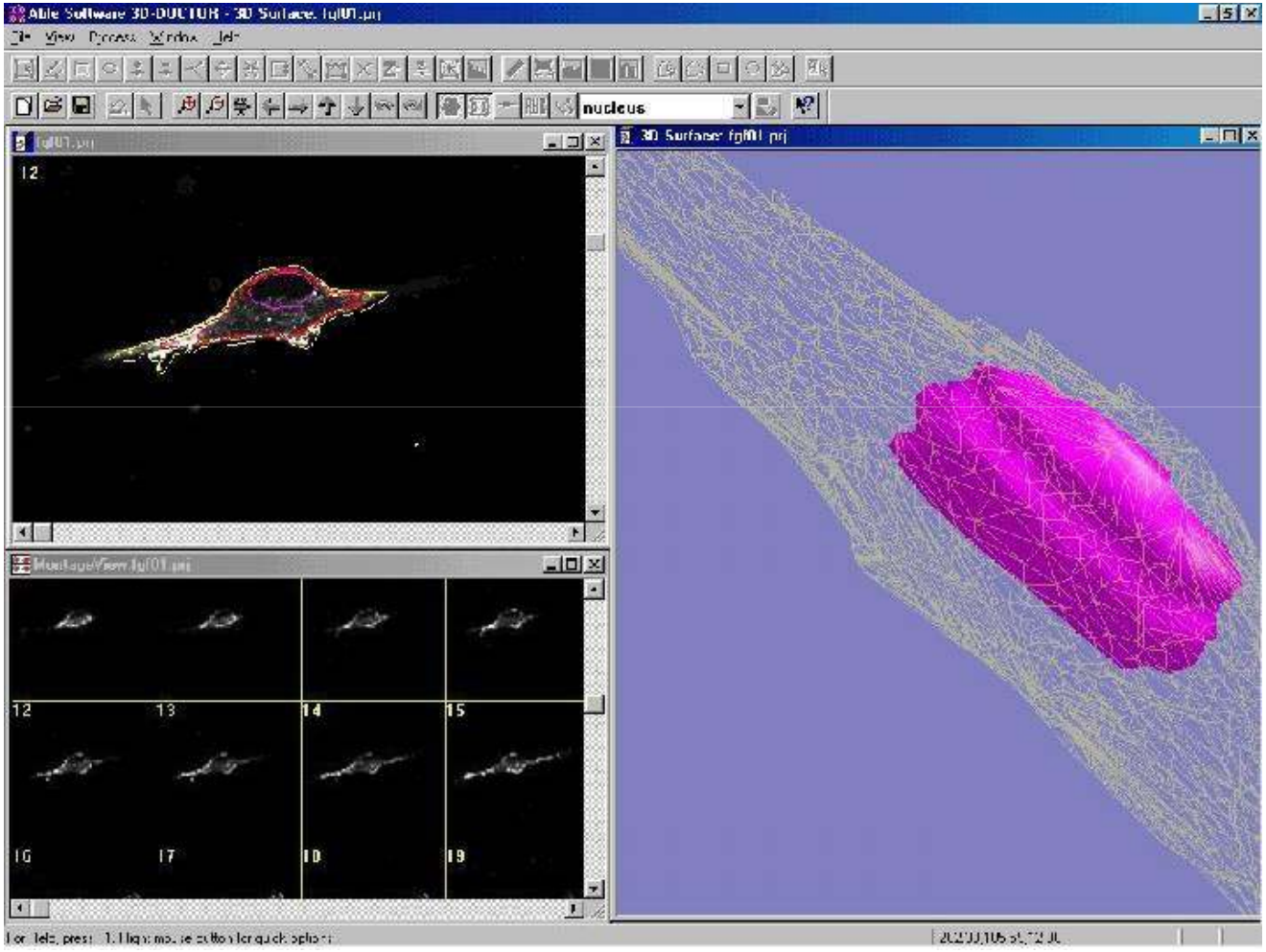
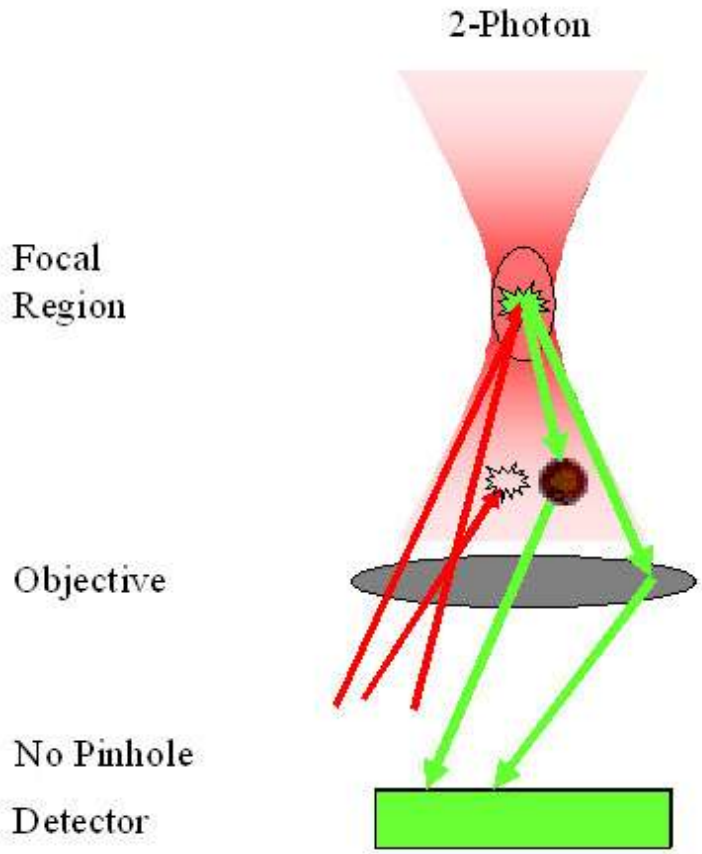
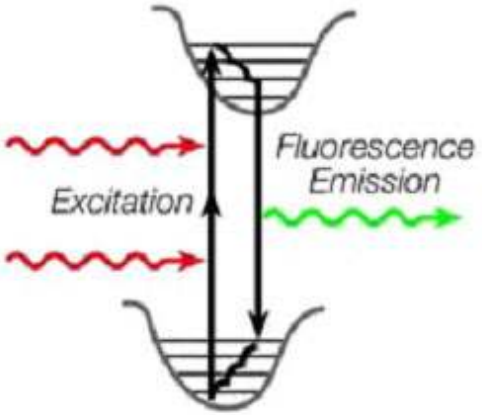


Image from www.be.caltech.edu/seminars.html

Two-Photon Microscopy





- 2 photons required for excitation
- No out-of-focus excitation
- No pinhole required
- Scattered light is detected

Image from http://research.stowers-institute.org

Confocal Imaging Example

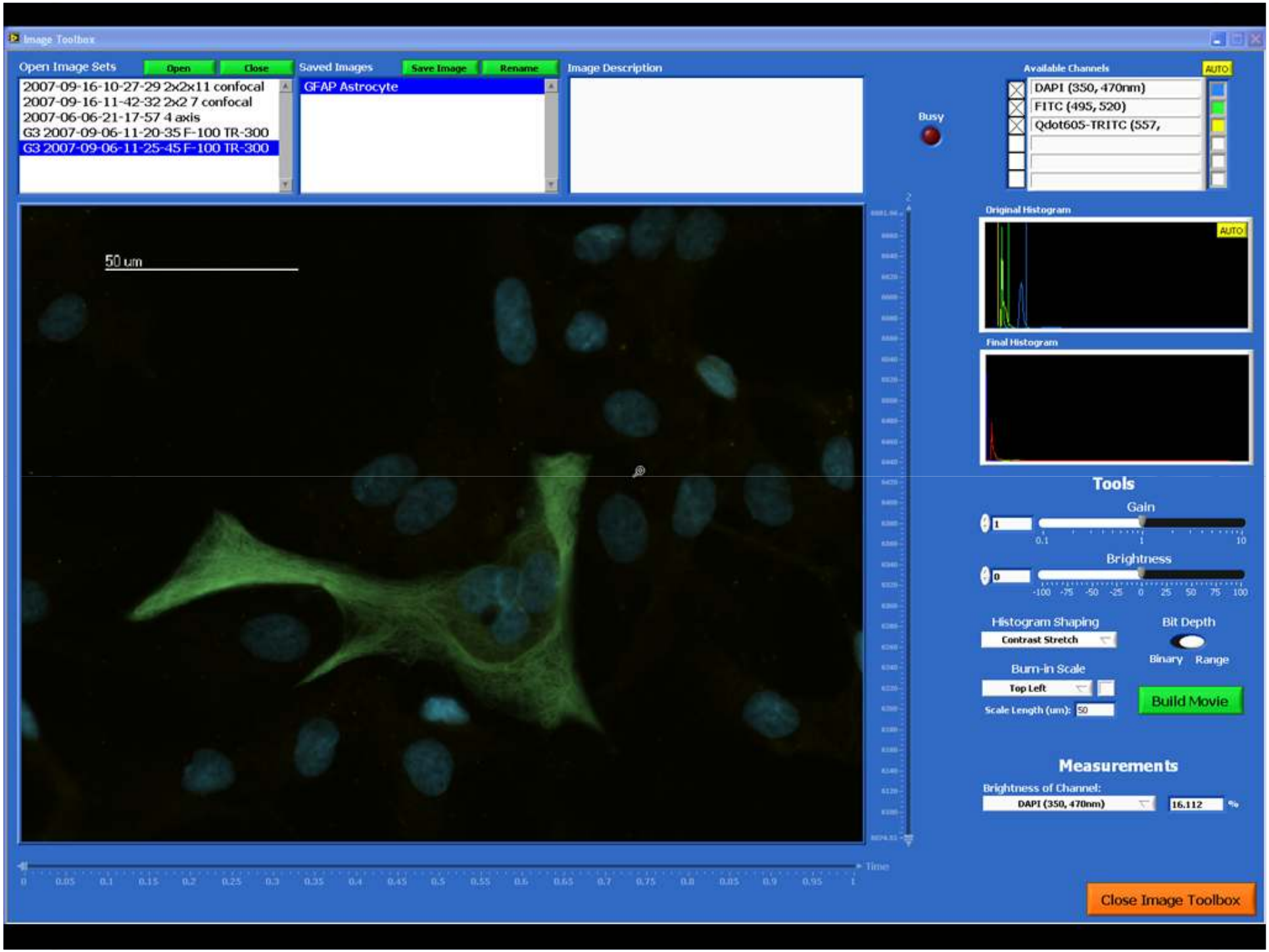


Image Toolbox
Open Image Sets
Open
Close
2007-09-16-10-27-29 2x2x11 confocal
2007-09-16-11-42-32 2x2 7 confocal
2007-06-06-21-17-57 4 axis
G3 2007-09-06-11-20-35 F-100 TR-300
G3 2007-09-06-11-25-45 F-100 TR-300
Saved Images
Save Image
Rename
GFAP Astrocyte
Image Description
Busy
Available Channels
AUTO
DAPI (350, 470nm)
FITC (495, 520)
Qdot605-TRITC (557,
Original Histogram
AUTO
Final Histogram
50 um
Tools
Gain
1
0.1
1
10
Brightness
0
-100 -75 -50 -25 0 25 50 75 100
Histogram Shaping
Contrast Stretch
Bit Depth
Binary
Range
Burn-in Scale
Top Left
Scale Length (um):
50
Build Movie
Measurements
Brightness of Channel:
DAPI (350, 470nm)
16.112
%
Time
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95 1
Close Image Toolbox

Acknowledgements

- **Thesis Defense Committee**
  - Dr. Gabriel Silva, chair
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  - Dr. Michael Heller
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  - Brent Runnels
  - Doug Shepard
- **Olympus America, Inc.**
  - Nicholas George
- **Silva Research Group**
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  - Diana Yu
  - Marius Buibas
  - Smita Pathak
  - Matt Li
  - David Kupec
  - Krystal Chao
  - Chris MacDonald
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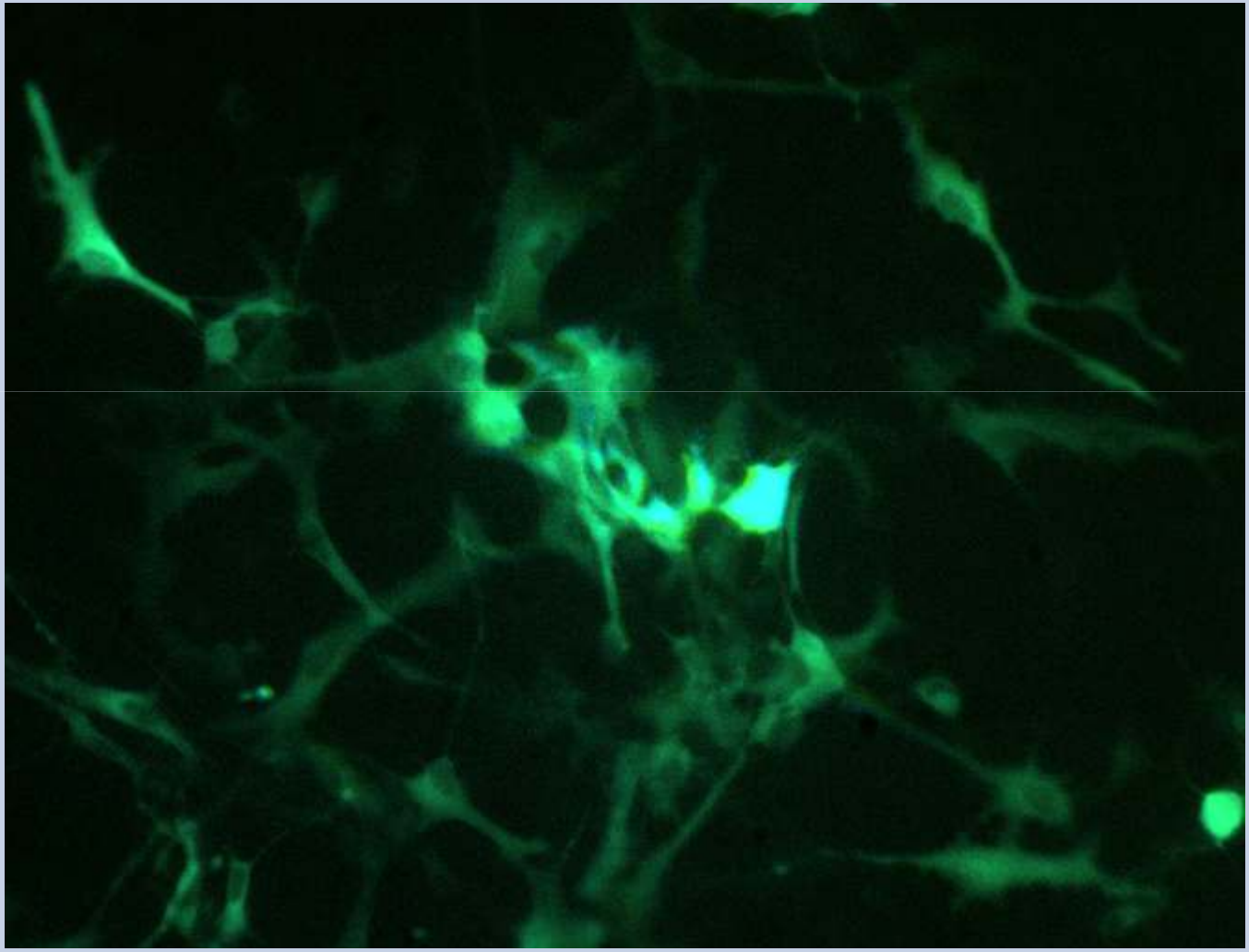
Questions?



Movie courtesy of Diana Yu, Silva Research Group

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