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

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Master’s Thesis Defense
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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
• Discussion
• Questions
• 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:
  - $[\text{Ca}^{2+}]$ Fluor-4 AM (white)
  - $[\text{Ca}^{2+}]$ 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

Olympus IX-81 Scope

Scion Stage Controller

Lambda DG-4

Sutter 10-3

Scope PC

LabVIEW

RS232

Firewire

1GB Ethernet

4-core Linux

ScopePC/LabVIEW

* Controls devices
* Records Images
* Sends images and scope data (position, magnification) info to 4-corePC in real-time

4-core PC

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
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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

Observation of current microscopy experiments
Meeting w/ Dr. Silva to discuss criteria
Development begins using LabVIEW
Olympus delivers proprietary commands
Meetings with scope users

Version 1.0.0

Version 2.0.0 presented to lab members

Version 3.0.0

Version 4.0.0

Version 4.3.0

Meeting w/ Brent Runnells

New hard drive installed
Meetings with scope users

Sutter 10-3 implemented

Sutter 10-3 optimized

Image Toolbox

Meeting with Jorge Noguera

Robustness testing and debugging
Auto-tiling added to Image Toolbox
Measurements added to Image Toolbox
Development Challenges

- Hamamatsu Camera
  - Driver conflict with ImagePro
  - Non-compliance 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

<table>
<thead>
<tr>
<th>Single Timestamp</th>
<th>Multiple Timestamps</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single imaging location</strong></td>
<td><strong>‘Still Snap’</strong> Record one image on each channel</td>
</tr>
<tr>
<td></td>
<td><strong>‘Still Movie’</strong> Repeat recording one image per channel with timestamps for a pre-defined period of time</td>
</tr>
<tr>
<td><strong>Multiple imaging locations</strong></td>
<td><strong>‘3-D Snap’</strong> Record one image on each channel, per image location</td>
</tr>
<tr>
<td></td>
<td><strong>‘3-D Movie’</strong> Repeat ‘3-D Snap’ recording, with timestamps, for a pre-defined period of time</td>
</tr>
</tbody>
</table>
‘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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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
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Questions?

Movie courtesy of Diana Yu, Silva Research Group
References


