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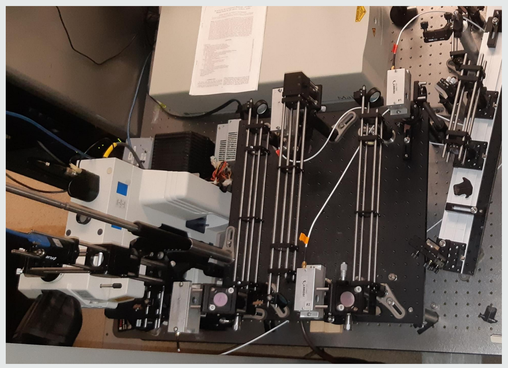
Software Control of Random-Access Acousto-Optic Deflector-Based Laser Scanning Microscope for Ultrafast Monitoring of Neural Network Activity

Introduction

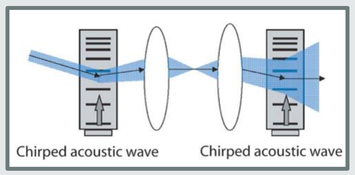
- The client for this project is Dr. Alan Fine of the Department of Biophysics at Dalhousie University.
- The research lab of Dr. Fine has constructed a system to perform Laser Scanning Fluorescence Microscopy (LSM) using acousto-optic deflectors (AODs). Unlike traditional galvanometer-based LSM systems, an AOD-based system allows for inertia-free laser scanning of samples.
- The objective of this project is provide a software package that enables the user to perform Raster Scanning, Fast Scanning of Regions of Interest (ROI), and time-series plotting of fluorescence intensity for each ROI.

Lab Hardware System



$$\theta = \frac{\lambda(f_{1c} - f_{2c})}{v}$$

$$F_{AOL} = \frac{v^2}{2\lambda\alpha}$$



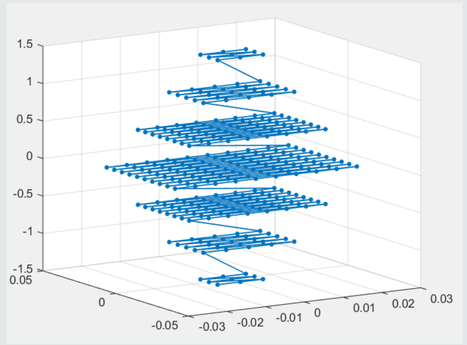
(1) Deflection angle, θ, depends on difference between the center acoustic frequencies in each pair of AODs.
(2) Focal length, F_{AOL} ,depends on the chirped rate.

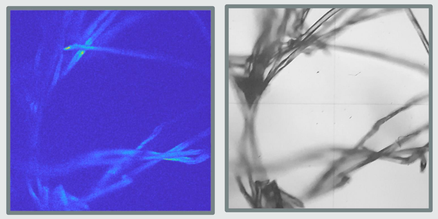
Design Process

- The first phase of the project was dedicated to researching the concepts involved. A fundamental understanding of LSM, neural imaging and current software was necessary.
- The second phase of the project has been dedicated to software implementation, testing and validation, and documentation.

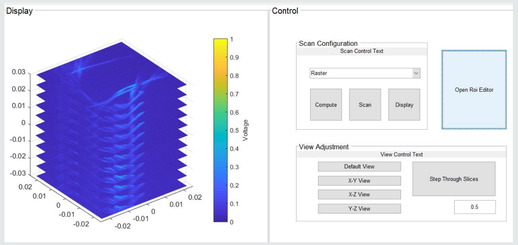
Raster Scan Results

- A raster scan consists of sequential line scans at specified depths, as shown below.
- The resulting data is sent back to the DAQ which is then displayed and processed by the software.

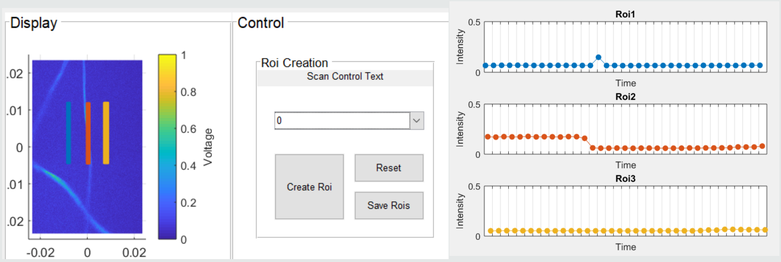




- A scanned plane is shown above (left) along with an image of the sample (right).
- The visible image stack and GUI of the software (below).



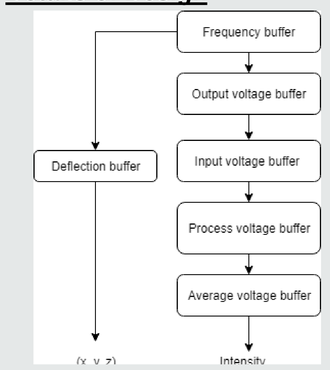
Fast Scan Results



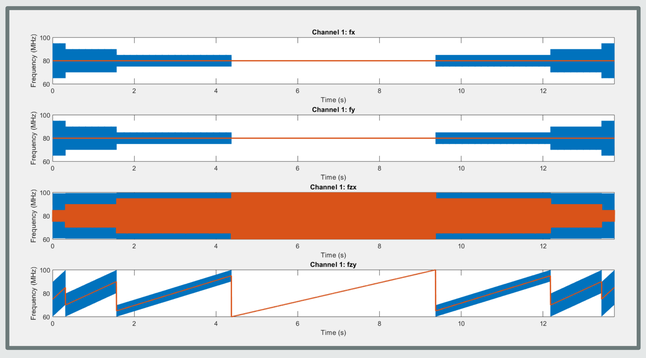
- Fast scanning allows scanning of selected regions of interest (ROIs) in the GUI (far left).
- In the test shown (left) movement of the microscope stage produces changes in the fluorescence intensity in each ROI indicated in the GUI (right).

- An AOD will produce a deflection angle that depends on the frequency of the RF signal that drives it. Therefore, the software calculates the required RF frequencies to produce the required scanning pattern. Given these frequencies, the software calculates the required input DC voltage to be sent to the Voltage-Controlled Oscillators (VCOs).
- The DC voltage buffer is sent to the VCOs while the fluorescence intensity is simultaneously received in the input buffer.
- The input buffer is processed and the intensity values are mapped on to coordinates (θ_x, θ_y) based on the frequencies that were used to produce the scan (see Equation 1).

Details of Design



Flow diagram of software output



Frequency output pattern for 4 channels

Conclusion

- A large portion of the software was rewritten to support the new version of MATLAB and DAQ toolbox.
- The basic necessary functionality has been implemented in the codebase, which consists of:
  - (1) Raster Scanning
  - (2) Fast Scanning
  - (3) Rectangular ROI Placement
  - (4) Time Series Plotting
- As a result of the re-implementation, improvements have been made to memory management, scan time and image quality.

Recommendations

- Replace the analog VCO with a digital oscillator board (DCO).
- Enhance ROI-specification and image capture features of the software.
- Use background DAQ operation features to stream output data in chunks, allowing for larger raster scans.

References

- Reddy, D., & Saggau, P. (2007). Fast Three-Dimensional Random Access Multi-Photon Microscopy for Functional Recording of Neuronal Activity. *Confocal, Multiphoton, and Nonlinear Microscopic Imaging III.* doi:10.1364/ecbo.2007.6630_45
- Reddy, G. D., & Saggau, P. (2005). Fast three-dimensional laser scanning scheme using acousto-optic deflectors. *Journal of Biomedical Optics, 10(6), 064038. doi:10.1117/1.2141504*