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Improving the Data Acquisition of the Dual-Wedge Confocal Microscope

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Abstract

A new method of generating images from the dual-wedge confocal microscope was developed. The previous method of data acquisition resulted in data rates of 100Hz. In addition, many data points were visited multiple times, while others were never visited. This caused the acquisition of a single frame to take more than half an hour. For collecting images of excised skin tissue to characterize the microscope, this is a major issue, as the shape of the sample changes over time.

To remedy this problem, a National Instruments Data Acquisition card was purchased, and signal conditioning circuitry was created to increase the data collection speed to collect every point visited in real time at the speeds at which the microscope is currently being operated. Images can now be acquired in seconds, and the capability exists to reach frame rates of 5 Hz., with faster scanners in the future.

The new acquisition technique had the added benefits of changing the analog to digital conversion resolution of the signal from 8-bits to 16-bits and improving the angular resolution from 500 to 1000 points per rotation. In the future there is the potential to increase the position resolution to 2000 points per rotation without replacing the encoders currently used.

Goals

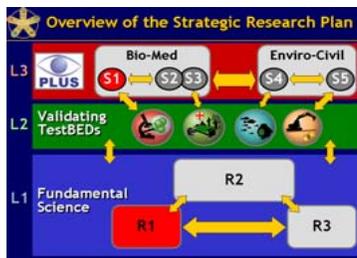
- Attain frame rates useable for imaging *in vivo* skin tissue, ultimately, at 5Hz.
- Increase ADC resolution and implement a tuneable dynamic range
- Add the ability to form an image with higher resolution

State of the Art

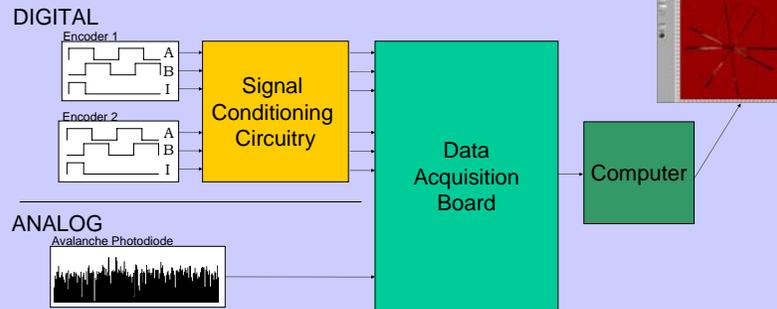
- Present: Point scanning confocal microscopes are complex, expensive and difficult to use.
- In-progress Research: Dual-wedge scanner may reduce the size of the scanning instrumentation, thereby simplifying the overall device and decreasing the cost, possibly lending itself to a hand-held design.

Technology Transfer Significance

- Skin cancer is one of the fastest growing cancers, with 1.2 million new cases diagnosed in the U.S. yearly
- Confocal microscopy has been shown to be useful in the detection of skin cancer margins, and clinical testing is being performed to determine its true potential for cancer detection
- Current commercial confocal systems are large and expensive; it may be possible to create a smaller, less expensive device using a dual-wedge scanner

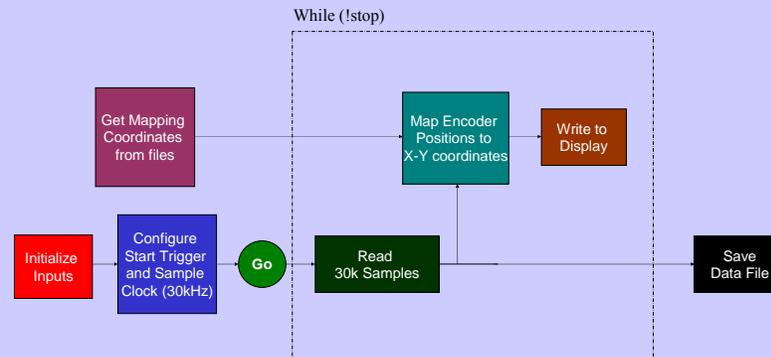


Readout Electronics Block Diagram



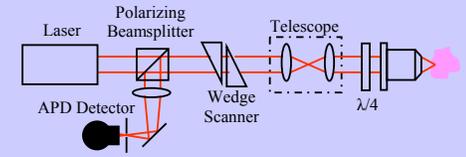
The above block diagram shows the implementation of the hardware used to capture the data. The digital signals from the digital encoders are amplified and filtered by the signal conditioning circuitry to remove unwanted noise from the system. The conditioned digital signals and the analog APD signal are passed to the National Instruments Data Acquisition Board, which provides a USB interface to the host computer running a program written in LabView that captures the data as each point is visited. The software then displays the image on the screen at a rate of 1Hz.

Software Implementation



The above shows the implementation of the software used to interface with the data acquisition hardware. Inputs for each encoder and the APD detector were defined using a common start trigger and sample clock to ensure that all signals were correlated and collected at the same time. Mapping functions to calculate x-y screen coordinates based on the two encoder values were found, and a table of values was generated using Matlab. This table is imported at the start of data acquisition. When 30k samples are collected, the software places the correct intensity values into a 2-dimensional matrix, which is then displayed on the screen. When the stop button is pressed, the software collects 1 million data points, then saves them to a file.

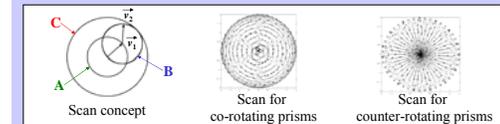
Concept of Dual-Wedge Confocal Microscope



The optical path begins at the laser, which has a strong P-polarized component, and then passes through a polarizing beamsplitter. The path continues through the dual-wedge scanner and through a telescope that properly aligns the scanner with the pupil of the objective. The path passes through a quarter-wave plate ($\lambda/4$), having an axis at 45 degrees to the laser polarization, thereby producing a circular polarized beam that is focused onto the sample by the objective. The incident light is then either absorbed, reflected, or scattered, with some of the light re-entering the microscope, passing through the objective, the quarter-wave plate, and the scanner. When the beam passes through the quarter-wave plate again, the plate has the same effect of a half-wave plate, switching the polarization from P-polarization to S-polarization. When the beam reaches the polarizing beamsplitter, the light is reflected toward the APD detector instead of toward the laser. The beam is focused by a tube lens through an aperture in a plane conjugate to the imaging plane, so only light that is scattered from the focus is received.

Scan Generation Using Dual-Wedges

The scanner consists of two identical prisms with their flats facing each other. When the beam passes through the first prism, it is refracted by some vector \vec{v}_1 , onto a point on circle A. The beam then passes through the second prism where it is refracted by vector \vec{v}_2 , onto a point on circle B. Varying the direction of \vec{v}_1 and \vec{v}_2 by rotating the prisms, any point within circle C can be reached. By rotating the prisms at different speeds, the two scan patterns shown below can be generated.



References

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