An FPGA based
Frequency Synthesiser for Acousto-Optic Lens Control

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Abstract: There is an increasing interest in the application of two photon microscopy in biology research. Current two-photon microscopes, which use galvanometer mirrors to steer the laser beam and build up an image, are too slow to monitor many fast spatially distributed physiological processes, such as neural activity. This paper describes the development of a system capable of generating the necessary signals to drive the four Acousto-Optic Deflectors (AODs), according to a host computer's requests. The system will lead to the development of an application-specific, high-performance Acousto-Optic Lens Control System (AOLCS) capable of arbitrary 3D scanning and compensate for inherent aberration of the optical systems as well as tissue induced aberrations, and revolutionise neural activity imaging.

1. Introduction

Fluorescence is the phenomenon at which a fluorophore absorbs a photon and emits a lower energy one. It is, however, possible for a fluorophore to simultaneously absorb 2 photons of half the energy normally required for fluorescent excitation. When the excited state decays there is again a photon emission [1]. This is the basic principle behind the operation of the two photon microscope. By taking advantage of this low energy excitation, two-photon microscopy has many advantages over conventional fluorescent imaging. Namely:

- The excitation wavelength can be in the near infrared (700-1000nm) where photo damage on tissue is much smaller [2].
- At these wavelengths absorption and scattering is also far smaller than higher on the spectrum, allowing imaging up to 1mm deep in the tissue [3].
- Two-photon absorption only happens at a significant probability under very high photon concentration (focal point). Therefore, no pinhole is necessary in the detection path of the microscope. Furthermore, the effects of photo damage and photo bleaching are spatially confined extending the viability of biological specimens during prolonged imaging [4].

By replacing the galvanometer mirrors with Acousto-Optic Deflectors (AOD) the scanning process of a two-photon microscope can be performed much faster. Their fixed speed (10-25μS), high precision control and pointing stability allow them to deflect a focused laser beam from one location to another rapidly and reproducibly. This enables random access multi-photon (RAMP) measurements to be made from multiple regions of interest (ROI) at high speed [5], [6].

A series of acousto-optic deflectors can form an Acousto-optic lens (AOL). Because of their fast electronically spatially controlled focal point, AOLs find applications in many fields of optics such as microscopy, holography, stereolithography, data storage etc [7]. In the field of two photon microscopy, they have the capacity to revolutionise instruments offering unparalleled speed and overcoming many of the shortcomings of their mechanical counterparts (for instance, galvanometric mirrors) since they are immune to mechanical inertia.

2. The Acousto-Optic Lens (AOL)

Acousto-optic or elasto-optic effects are terms describing the change of the refractive index of a medium caused by ultrasonic pressure waves or some other form of mechanical stressed applied to it. High efficiency AODs for two photon applications are usually made of tellurium dioxide crystals[8].

An acousto-optic device consists of a suitable crystal coupled with a piezo-electric transducer and sometimes an acoustically absorbing material on the end side to moderate reflections (figure 1a). When RF frequency is applied to the transducer the system acts like a "phase grating". Light propagating through it experiences a higher value of refractive index and therefore a lower wavefront velocity at regions with higher stress. Therefore, a narrow collimated beam of light incident upon the device is scattered into primary diffraction orders [9]. The diffraction of the beam is directly associated with the diffraction grating set up by the acoustic waves which in turn is dependant to the RF frequency that drives the transducer. Varying the frequency with time (chirping) fast enough can, therefore, cause the optical wavefront to curve bringing it to a line focus (figure 1b).

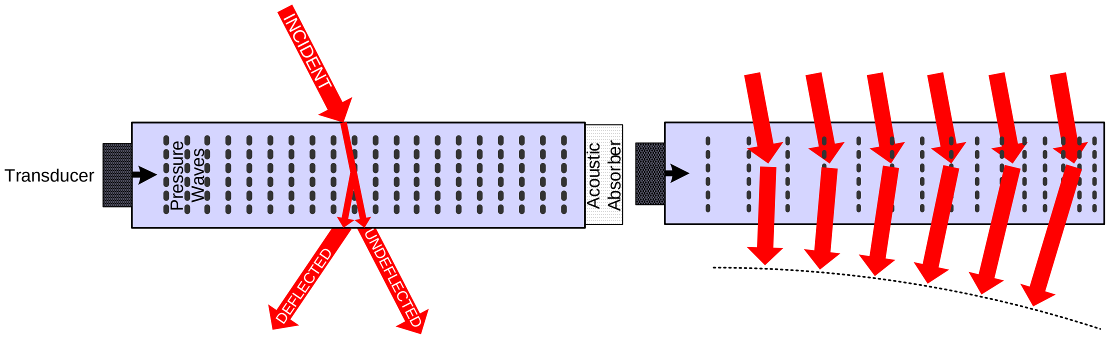

Figure 1. (a) A typical acousto-optic deflector. The transducer on the left converts the RF signal into a sear mode sound wave inside the crystal. The laser is diffracted because of the refractive index variations as different pressure regions depicted as black dashed lines are formed in the lattice. (b) An acousto-optic cylindrical lens formed by the different density of high pressure areas throughout the crystal. The chirped sound wave propagates across the crystal moving the focal plane at the speed of sound in TeO_2. A linear frequency ramp produces a perfectly circular wavefront.

In order to achieve a stationary focus a second AOD with a counter propagating wave or an inverted frequency ramp can be used to cancel out the acoustic gradient propagation across the crystal.

The acoustic ramp characteristics controls the focal line position of the cylindrical lens; changing the slope of the ramp moves it on the Z axis while changing the separation of the two ramps moves it on the X axis.

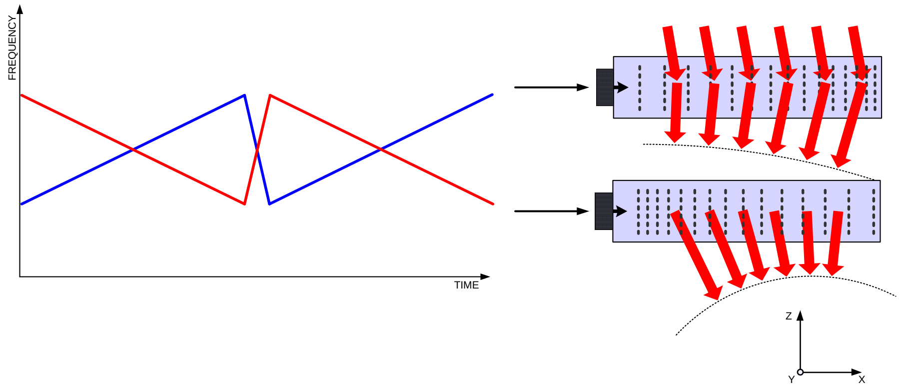

Figure 1. A second AOD with an inverted input (or counter propagating acoustic front) can be used to counter-act the effect of sound propagating through the crystal. This produces a cylindrical lens with a stationary focal point and at the same time cancels out the chromatic aberration at the centre of the field of view. Varying the slope of the input frequency ramps varies focus on Z while separation affects X axis.

Introducing another pair of counter propagating AODs orthogonal to the first enables the shaping of the wavefront in two axes, effectively constituting a diverging or converging spherical lens (figure 2), which can be used to focus to a point in the 3D space above and below the natural focal plane of a subsequent fixed lens system[10].

3. System Requirements

The target specifications set by the Silver Lab [11] after considering the neuro-scientific requirements were:

- Image with better than 1×1×2 µm resolution on X,Y,Z axis respectively.
- Image in 250×250×250 µm volume of tissue (corresponding to 7.8 million voxels when using the above resolution).
- To be able to focus the laser beam on 30 randomly chosen locations in the 3D space per millisecond.€

For these, the AOL control system should be able to generate the four separate, frequency and amplitude modulated, periodic signals that are required to drive the four AODs, given the appropriate record information. The output frequency range should be from ideally from 0 to 100 MHz -minimum requirement: 25 to 50MHz- with a rapid response to cope with steep ramp rates and returns to initial frequency. The MiniScan duration should be variable from 10 µs to 100 µs.

The system should be able to operate in at least four different selectable modes:

1. *SingleFrame* or *LiveScan* in *GridMode*: the AODs focuses on every voxel to construct an image
2. *SingleFrame* or *LiveScan* in *ScanMode*: the AODs scan in lines to construct an image
3. *Z-Stack* of 100 planes loaded on a permanent memory: the AODs continuously scan through the stored planes while the system remains off-line
4. *PointMode***:** AODs focuses on and continuously scans only some chosen points-ROI to monitor activity.

4. The prototype Acousto-Optic Lens Control System (AOLCS)

Dictated by the system requirements and after careful consideration of various components, tools and techniques for the development of the AOLCS, a selection was made,.

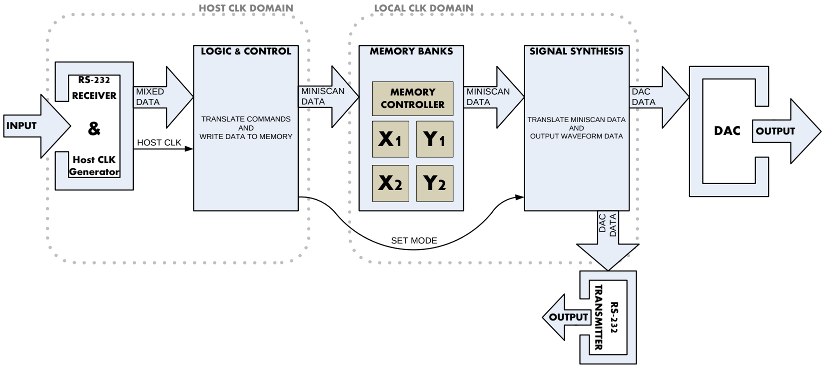

Figure 2. Block diagram of the implementation of the prototype AOLCS

An asynchronous serial link, based on the RS-232 standard, over an USB bridge was chosen for the link to the microscope's host computer because of its wide availability and simplicity. Two UART modules were implemented in VHDL on a low level definition, one for the receiver (RX) and one for the transmitter (TX). It was possible to successfully operate these modules at a 3 MBaud (3MB/s) which is the upper speed limit of the on-board USB to UART bridge (FT2232D).

It was decided for the logic and processing of the AOLCS to be implemented on an FPGA. Unlike a microcontroller an FPGA can perform multiple tasks in parallel. This translates to accurate timing of each process and meeting the time constrains set by the specifications even when using a relatively slow clock of 200MHz.

The core of the system is composed of three main modules, distinguished by the different task they are set to perform: The front end or input control module, the memories and the output control module.

The *input control module* is responsible for accepting and processing the control words and data sent by the host computer. It operates on a clock signal generated by UART RX module. It is therefore continuously in synchronisation with the host computer. It controls a state machine, consisting of enough states to cover the system's different mode requirements as well as debugging and verifications modes.

The *memory bank* consists of four single port RAM modules, each one holding the miniscan data –the data required to generate a frequency ramp- for one AOD. The RAM modules are 960 entries long and 96 bit wide allowing scanning at a theoretical maximum 960x960 horizontal resolution plane.

The *output control module* consists of a ramp control sub-module and the Direct Digital Synthesiser itself. The ramp control is responsible for reading the miniscan data provided by the Output Control module and when triggered, beginning with the tuning word which represents the starting frequency, feed the DDS with a constantly varying by ±Δf tuning word until the stop frequency is reached.

5. Results

In the current implementation of the AOLCS signal synthesiser module, a 32 bit accumulator operating on a 200 MHz clock was developed. Only the 8 Most Significant Bits (MSB) of its output were used, matched with 64 by 8 bits quarter sine wave LUT stored in distributed ROM. The frequency resolution of this configuration is 0.046 Hz which more than satisfies the requirements. Furthermore, the system is capable of hoping between frequencies in a rate equal to the frequency of the system clock, also satisfying the ramp rate requirements.

The truncation of the 24 LSB in order to reduce the LUT size to a practically achievable one, causes phase modulation of the output sinusoid, introducing non-harmonic distortion – spurious products. The Spurious Free Dynamic Range (SFDR) for more than 4 truncation bits is given by (1) [12]:

$$SFDR \approx -6.02 \times P\ dBc \quad (1)$$

where P is the number of truncated bits. The SFDR of the implemented DDS was calculated to be 48 dBc.

The output of the LUT is the output of the Direct Digital Synthesiser and is directly fed to the parallel DAC at the falling edge of its clock.

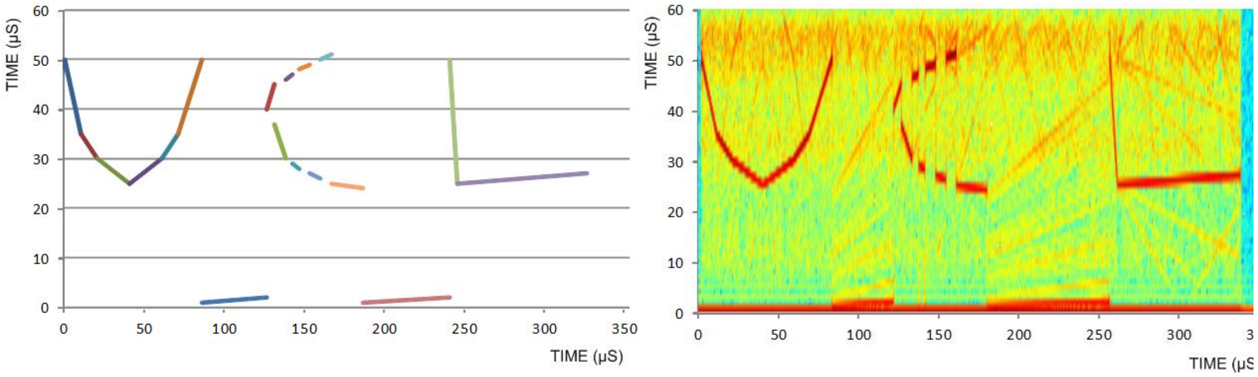

Figure 3. Demonstration of system behaviour (DDS agility)
(a) Plotted of expected Miniscan (b) Spectrogram of actual generated signal

6. Future Work

Further development of the system could include non-linear frequency ramp generation to allow fast scanning in all three dimensions. In addition, phase dithering or Taylor series correction could be implemented in order to improve the SFDR of the synthesised signal. Finally, the link between the system and the host computer could be revisited, to eliminate the latency.

7. Conclusion

This paper has briefly discussed the design and development of an AOCS for a random-access two photon microscope. The chosen techniques and technologies were cited and the operation of the system was described in short. The system, although not yet integrated with the instrument, performed as expected electrically, producing satisfying results. Future improvements were also considered.

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