

An Electronic Control and Image Acquisition System for Laser Scanning Microscopy

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Abstract

This paper presents an electronic system that controls the entire operation of a laser scanning microscopy system through a DAQ card. Proposed system does not only create the required electro-coil driving signal peculiar to magnetically actuated micro-scanner that enables the raster-scanning movement, but also is responsible from the image acquisition part by both serially gathering the laser intensity data and using it to construct a meaningful microscopy image. Micro-scanner which is fabricated using Ni as the structural material is utilized in the system. The micro-scanner's slow and fast scan frequencies are measured to be 250 Hz and 1560 Hz, respectively. Model of the DAQ card used in the system is NI-6356 which has maximum 5 mA current and 10 V voltage outputs. A power amplifier circuit with LM 386 is designed and added to the system for increasing field-of-view of the micro-scanner. The operation of the proposed system is demonstrated by acquiring data and constructing images from the USAF resolution target.

1. Introduction

Laser scanning confocal microscopy (LSCM) is one of the most suitable methods for imaging/early diagnosis of cancer cells since it is cheaper, preferably portable and performs without any harmful radiation together with its ability doing real-time in-vivo imaging [1]. Most importantly, LSCM allows high-resolution imaging, while at the same time its optical sectioning ability and in-depth imaging about a few hundred micrometers beneath the tissue surface enable precise cancer diagnosis on the tumor sites [2–5].

In this work, an electronic system that controls the entire operation of an LSCM system through a DAQ card is proposed. This system does not only create the required electro-coil driving signal, peculiar to magnetically actuated micro-scanner but also is responsible from the image acquisition part by both serially collecting the laser intensity data and transforming it to a meaningful microscopy image. As it is depicted in Fig 1, at the heart of the implemented miniaturized laser scanning confocal microscope, there is a gimballed two-dimensional (2-D) electromagnetically actuated micro-scanner with a large total optical scanning angle and field-of-view. To be able to actuate this micro-scanner which is fabricated using Ni as the structural

material, one should drive the miniaturized electro-coil in very precise fashion. To ensure this, in the first part of this work, a Matlab code that allows user to choose various signal waveforms and values, is developed along with its graphical user interface (GUI). Subsequently, a driving circuitry consisting off-the-shelf components is designed to satisfy the power constraint for the actuation system. In the second part of this proposed work, as it is shown in Fig 1, the target is raster scanned in real-time using a 653nm laser with the help of magnetically actuated micro-scanner. The reflected light from the resolution target is passed through the collimation optics and eventually collected in the photomultiplier tube (PMT) and transmitted to the NI-6356 DAQ card. Collected data is then processed in developed Matlab software and the respective image is formed. In this paper, the implementation of the proposed electronic embedded system consisting of (1) a coil driving part and (2) an image acquisition part will be discussed in detail.

2. System Description

The main target of the proposed system as it is depicted in Fig.1 is to basically get a 2-D image of a target by raster-scanning it with the help of a micro-scanner. In the proposed system, a DAQ Card controlled by a Matlab program is utilized both for generating the actuation signal and constructing an image simultaneously.

To accomplish scanning, the micro-scanner should make an orthogonal movement in slow and fast scan directions. The micro-scanner is designed to have some specific fast and slow-scan resonant frequencies which should be precisely triggered. Hence, the actuation signal must contain both slow and fast scan resonant frequencies in order to result in an orthogonal movement. To generate such an actuation signal, a function generator program is written on Matlab. Waveform, amplitude and frequency of the actuation signal could be defined from graphical user interface (GUI) of the function generator program. The actuation signal is then generated by the DAQ card using these specifications. These specifications can be used to control and adjust the motion of the micro-scanner. To actuate micro-scanner, besides precise fast and slow scan frequencies, sufficient power should be applied to the micro-scanner for maintaining appropriate field-of-view (FOV). The DAQ Card's maximum output current is 5 mA; and, the maximum output voltage is 10 V. Therefore, it can produce 50 mW maximum power; and, this power does not suffice to obtain an orthogonal scanning movement. Hence, a power amplification circuit

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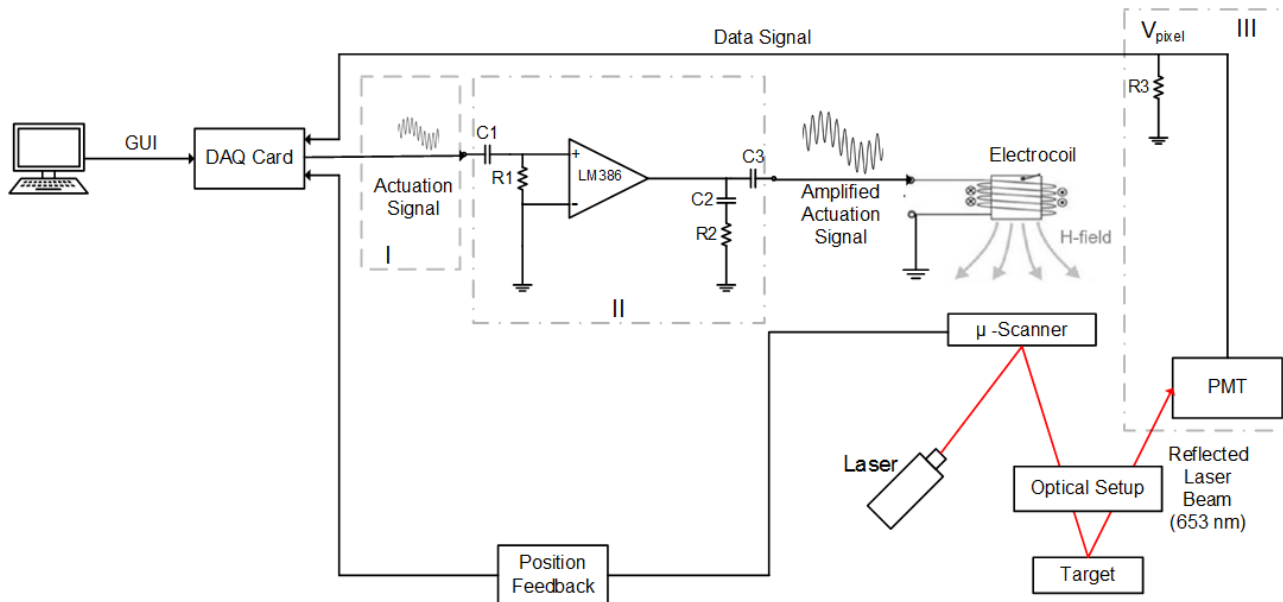


Figure 1. Block diagram of the proposed system

is utilized at the analog output channel of the DAQ card. In this circuit LM386 is used as power amplifier. Amplified actuation signal is directed to an electro-coil which will cause actuation of micro-scanner by generating magnetic field.

The actuated micro-scanner scans the target in the direction of fast and slow scan axes by reflecting a laser beam to the target through an optical setup. Laser beam reflected from the target is collected by a Photomultiplier Tube (PMT) after passing through the optical setup. The PMT generates a current depending on to the intensity of reflected laser beam. The current value is converted to voltage value by using a resistor (R3) connected to the output of the PMT. The voltage value (V_{pixel}) on the resistor R3 is acquired by the DAQ Card; and it is converted a pixel intensity value between 0 and 255. This conversion is done according to calibrated maximum and minimum voltage values. After all of the pixel values gathered in this manner, a 2-D image is constructed and displayed on the GUI of the Matlab program. As a result, both actuation of the micro-scanner and construction of the target image can be controlled on the Matlab program.

3. Actuation of the Micro-scanner

A Ni material based micro-scanner which can be actuated by a varying magnetic field is utilized in the system. Thus, electromagnetic actuation is chosen as the actuation method. In this method, according to the power of the magnetic field, micro-scanner's field of view (area scanned by micro-scanner, FOV) changes. Field of view of the micro-scanner expands with greater magnetic field because movement of micro-scanner is linearly dependent with magnetic field. To create greater magnetic field, current applied to electro-coil should be increased according to Ampere's Law which can be expressed as;

$$B = \mu n I \quad (1)$$

where B is the magnetic field strength, μ is the permeability of the core material, n is the turn density of the coil, and I is the amplitude of the driving current signal. In respect to

(1), magnetic field is linearly dependent with permeability, turn density and current. The permeability and turn density values are material dependent; and, they are set by the coil used in the setup. To get adequate field of view for micro-scanner of this setup, a minimum driving current value of 100 mA should be applied to the electro-coil. A power amplifier circuit is added to the output of the DAQ card, since the maximum output current value of the DAQ Card is measured to be 5mA which is not sufficient to drive electro-coil. To amplify the output current of the DAQ Card, an amplification circuit with LM 386 power amplifier is added to the setup. LM 386 is used as the amplifier due to its wide range of gain, which can be adjusted between 20 and 200 by connecting a proper resistor between pins 1 and 8. It can operate between 4V-12V DC supply voltage; and, it also has low quiescent current consumption which is 4 mA. The amplifier circuit is adjusted to have the gain of 20, since the DAQ Card's output current is 5 mA. Also, 12V is used as DC supply. Amplifier circuit has low pass filter which is composed of R1 and C1 to filter out undesired high frequency input signals that can trigger unwanted modes of the micro-scanner. The low scan and fast scan frequencies of the micro-scanner utilized in the setup are measured to be 250 Hz and 1560 Hz, respectively. Moreover, a Boucherot cell which is composed of C2 and R2 is used in the circuit to damp high frequency oscillations and provide stability for the output signal. A DC Block (C3) capacitor is used for blocking the DC component of the output signal. The resulting AC signal is coupled to the electro-coil to obtain the desired raster-scanning movement.

4. System Implementation

The DAQ Card is used to generate actuation signals and acquire voltage values which are converted from output current values of PMT. In order to control DAQ Card, a GUI shown in Fig. 3a is designed and programmed on Matlab software. Flowchart of the Matlab program is shown in Fig. 2. Matlab program executes signal generation firstly, and then according to the specified signal parameters, image acquisition is ex-

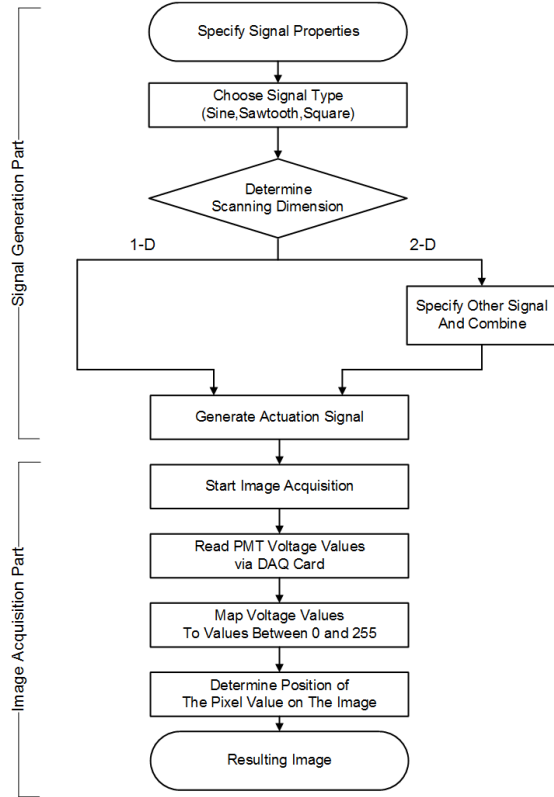


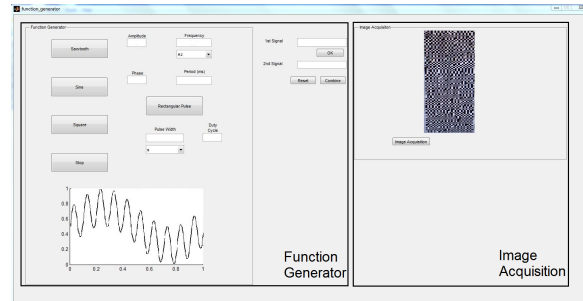
Figure 2. Flowchart of the Matlab program

ecuted. These two processes are displayed on the GUI as two main blocks; and they are placed on the left and right separately.

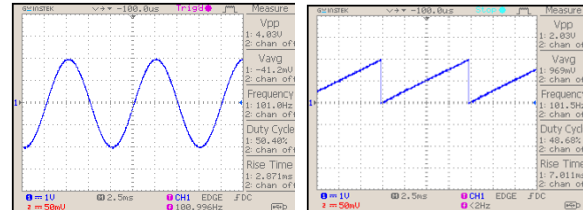
The left block is the actuation signal generating part, where the right block is the image acquisition/display part. The amplitude and frequency of the actuation signal can be specified on the left block. The actuation signal can be generated in the form of sinusoidal, saw tooth and, square wave as shown in Fig.3b, Fig.3c, Fig.3d, respectively. The actuation signal is created in Matlab as a matrix by using Matlab built-in commands. Sampling rate of 3 million samples per second is used for the signal generation; i.e. in order to scan target for 2 seconds, a matrix which is 6000000-by-1 (m-by-n) is created. The matrix is delivered to the DAQ Card's output by using commands in Matlab Data Acquisition Toolbox. Actuation signal with one harmonic can be used for actuating the micro-scanner in 1-D (along fast scan axis or slow scan axis). To start actuation in 2-D (along both fast scan axis and slow scan axis), the user needs to combine two signals with different frequencies. In combining part of the signal generating left block, the user can combine two different signals and generate an actuation signal with two harmonics to actuate the micro-scanner in 2-D. An example 2-D actuation signal which contains two sinusoidal signals at 100 Hz and 1 kHz is shown in Fig.3e. In the same manner, different types of electromagnetic actuation can be achieved by combining different signal types with specific amplitude, shape, and frequency parameters on the GUI.

The right block of the GUI is used for the image acquisition and image analysis. The image constructed using acquired signals is displayed on this block. The user can start data acquisition process by clicking image acquisition button in the right block. When image acquisition process is started, voltage val-

ues which are converted from output current value of PMT by R3 resistor are acquired by DAQ Card in serially through single analog input channel. A calibration method is applied to determine the pixel value of the acquired voltage value. In the calibration process, the voltage value V_{pixel} is converted to a value between 0 and 255, where 0 value represents black and 255 represents white color. The converted value is a pixel for the constructed image. When the process is completed, the final image is displayed on the right block of the GUI. In the experiments USAF resolution target is used. The scanner is directed to the three sets of lines with different widths which are present on the USAF resolution target. Obtained 1-D scanning signal from the scanning process and the constructed image regarding to this signal are shown in Fig. 4a and Fig. 4b, respectively.

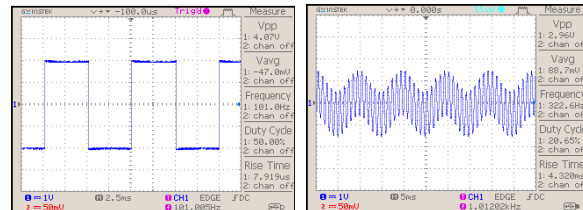


(a) GUI on Matlab



(b) Sinusoidal wave

(c) Saw tooth wave



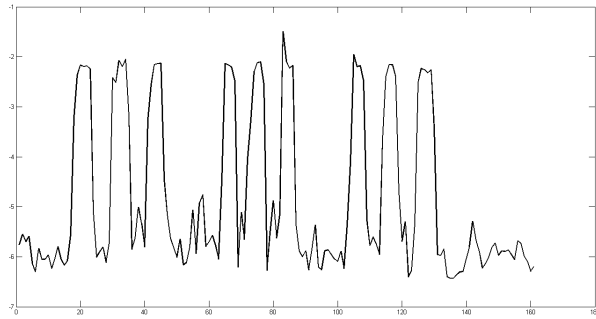
(d) Square wave

(e) Sine wave with 2 harmonics

Figure 3. GUI on Matlab and example waves generated by DAQ Card with $f = 100$ Hz and $V_{pp} = 4$. Harmonic wave is composed of 100 Hz and 1 kHz sinusoidal wave

5. Conclusion

In this paper, a confocal microscopy system with micro-scanner which is controlled by a DAQ Card is presented. Purpose of the system is to scan an object with micro-scanner and display it on a designed GUI which is programmed on Matlab software. The system consists of two parts namely; actuation signal generation and image acquisition parts. In the actuation signal part, an actuation signal is generated from the GUI to create a magnetic field which results in a scanning motion of the micro-scanner. On the GUI, various parameters can be set and



(a) Plot of acquired 1-D scan data



(b) Constructed image from the acquired 1-D scan

Figure 4. Experimental results from a 1-D scan applied to the USAF resolution target

different types of signals can be combined to generate different types of actuation signals. The current supplied by the DAQ card is amplified to 100 mA for creating sufficient magnetic field for actuation of the micro-scanner. Therefore, a power amplifier circuit with LM 386 integrated circuit is designed and added to the output of the DAQ card analog output channel. The electro-coil placed near to the micro-scanner is driven with the amplified actuation signal. In the image acquisition part, a PMT collects laser beams which are reflected from the scanning target; and it generates electrical current values according to power of the reflected laser beams. The electrical current values are converted to voltage values by a resistor. Converted voltage values are acquired by DAQ Card in a serial manner. The acquired voltage values are mapped between 0 and 255. According to the dimension information of the scan, an image is constructed by gathering all of the pixels belong to the image. Successful operation of the proposed system is demonstrated by constructing images from USAF resolution target.

6. References

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