Scan Software

1 Introduction

Scan is a graphical user interface designed to control a confocal microscope BIORAD MRC-600. The software can acquire simultaneously two channels, scanning an area of $0.4 \times 0.3mm$. Several integrated functions allow the user to focus the microscope, grab images or sequences of images, perform line scanning, zoom in a specific region, and apply Kalman filter to the images. In addition, the software can control the stage position and amplifiers setup.

The software runs on the Matlab v.7 environment. The data acquisition is performed by a 12-bit NI PCI-6110 board.

The first part of the manual, Section 2 to 4, gives general information about the software. Any general user should read these sections. The appendices give more detailed information, for advanced user trying to modify the code or assemble a new system.

2 Requirements

This section lists and describes the software and hardware required to run *Scan*.

2.1 Software

- **MATLAB:** Matlab v.7 or later with the Data Adquisition Toolbox (v.2.0 or later).

2.2 Hardware

- **Acquisition Board:** a National Instruments data acquisition board NI PCI-6110 with its drivers.
- **Computer:** a computer equipped with a Pentium 4 Processor 2.4GHz with 1GB RAM memory, and Windows 2000 (or later version) operating system. Theoretically, *Scan* should run on any operating system supporting Matlab v.7, e.g. Linux. However, this feature was not tested.

- **Amplifier:** two current amplifiers SR570 from Stanford Instruments. Notice that other amplifiers may be used; however, the user will not be able to control them from the *Scan* software.
- **Stage Control (optional):** a stepping motor CMA-25PP, a controller ESP100, and a joystick ESP300J from Newport Corporation.
- **Complementary Hardware:** several connectors, cables and accessories are needed to interconnect the confocal microscope, the amplifiers and the stepping motor with the computer. A detailed hardware list and instrucction about the wiring is given in Appendix C.

3 Installation

This section explains how to install and configure the *Scan* software and related components.

3.1 Installing the Acquisition Board

Install the drivers of the data acquisition board in the computer, and then the board. Refer to the board manual for more information on the installation procedure. Run the "Measurement and Automation" software to check the correct operation of the board and observe the device number. This number identifies the board when more than one National Instrument board have been installed.

3.2 Installing Matlab

Install Matlab and its toolboxes following the instructions of the Matlab manual. Run the instruction `daqhwinfo('nidaq')` from the command line to check if Matlab recognizes the installed NI board. You should be able to read:

```
AdaptorDllName: 'C:\MATLAB6p5\toolbox\daq\daq\private\mwnidaq.dll'
AdaptorDllVersion: 'Version 2.2    (R13) 28-Jun-2002'
AdaptorName: 'nidaq'
BoardNames: {'PCI-6110E'}
InstalledBoardIds: {'1'}
ObjectConstructorName: {'analoginput('nidaq',1)'
        'analogoutput('nidaq',1)' 'digitalio('nidaq',1)'}
```

3.3 Installing *Scan*

To install the software, unzip the `scan.zip` file. When it is decompressed, automatically, a folder called Scan with all the files will be created in the root of the hard drive. Before you can run the software, you must set up the Matlab path and the *Scan* software.

Setting Matlab Path

In order to enable Matlab to find the *Scan* software and run it, you have to define the path to the directory which store the files. Go to the **File** menu in the Matlab window and select **Set Path**. Then, add the Scan directory.

Configuring *Scan*

Scan reads the `start.m` file during the starting process. This file defines several variables related to the hardware and default parameters. You can edit this file using the Matlab editor or any other text editor, such as Notepad. You must edit this file to match the hardware installed in you computer. The first four variables are defined as:

```
handles.BoardID=1;
handles.PAmp1=1;
handles.PAmp2=2;
handles.PStage=3;
```

The variable `handles.BoardID` represents the device number discussed in Section 3.1. The next three variables represent the communication serial port for each piece of equipment conneted to the computer, i.e., the amplifiers and the controler for the stepping motor of the stage. The valid arguments are integer numbres form 0 to 6. Use 0 when the equipment is disable or it is other than the suggested in Section 2.2. Use 1 to 6 to represent the port in which it is connected, e.g. `handles.PAmp1=1` means that the amplifier of Channel 1 is on port COM1.

Notice that this file defines other parameters, such as the position of different windows and the default parameters for the amplifiers.

3.4 Running *Scan*

After opening Matlab, type and execute `Scan` from the command line to start the *Scan* software. Another option is to copy the Scan shortcut from the Scan directory to the Desktop. Double-clicking on the shortcut with the mouse opens Matlab and launchs the *Scan* software.

4 Using *Scan*

Scan consists of several graphical user interface windows that allow the user to control the confocal microscope. This section presents the windows, functions and variables in relation to the image acquisition process.

4.1 Main Window

After starting *Scan*, the main window is launched (see Fig. 1). This window consists in two image displays, one for each channel, several buttons to performs different actions, and menus.

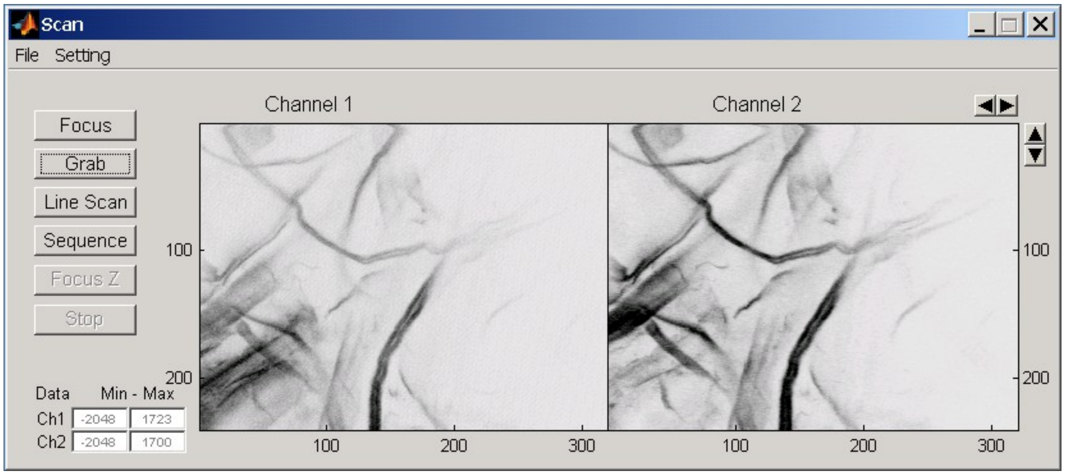

Figure 1: *Scan* main window.

4.1.1 Displays

The main window has two images displaying a preview of the channels. Each image is 320 pixels wide by 240 lines height. The main purpose of these is to show a preview of each channel, help to focus the microscope, and select the areas of interest where the user may want to zoom-in.

4.1.2 Menus

In the top left side of the main window there are two pull-down menus: **File** and **Setting**. The order in which the menus are shown (from left to right and from top to botton) suggests the logic order in which they should be used. **File** allows the user to select the working directory, load and save the configuration. From **Setting**, the user can open different configuration windows, as well as enable/disable several functions.

File Menu

- **Select Directory:** lets the user to select the working directory for Matlab, where images will be saved.
- **Load Parameters:** retrieves parameter from old experiments, previously saved.
- **Save Parameters:** saves to a file the configuration parameters requiredby the software to scan images, such as amplifier parameters, size of the image, etc.
- **Load Color Map:** load a differet colormap table to display the data. *Scan* provides three colormap tables: gray, green and rainbow. Gray is loaded by default. Refer to Appendix B in order to create customized colomap tables.

Setting Menu

- **Amplifier:** opens a new windows which lets the user to set up the amplifiers; refer to Section 4.2 for more information.
- **Configuration:** opens a window which lets the user to set up the scanning process by chosing parameters such as size of the image, the integration factor, the zoom-in factor, etc. Refer to Section 4.3 for a detailed list of all the fields in this window.
- **Stage:** opens a window which lets the user to control the position of the stage; see Section 4.4.
- **Channels:** selects the channels from which data will be acquired, displayed and/or saved.
- **Kalman Filter:** enable the Kalman filter. The number of images being averaged is defined in the Configuration window.
- **Stage Shift:** enables the stage motion when a sequence of images is being acquired. The step size is defined in the Configuration window.
- **Focus Zoom-In:** enables the user to focus the microscope when a selected area is being scanned. When this item of the menu is selected, the main window is extended downward showing an enlarged view of the selected area (see Fig. 2). Refer to Section 4.1.4 to learn how to select an area of interest with the mouse.

4.1.3 Buttons

The buttons located in the left side of the main window trigger the acquisition process in different modes. Only one mode a the time can be executed; thus, the other buttons are diseabled when they are not selected.

- **Focus:** initiates the scanning process. The images are acquires continuously and displayed in the main window. While this process is running, the user can focus the microscope using the Amplifier and Stage windows. Use the Stop button to stop this process.
- **Grab:** grabs one image based on the parameters of the Configuration window. The image is displayed in a separated window; see Section 4.5.
- **Line Scan:** scans many times a selected line based on the parameters of the Configuration window. Refer to Section 4.1.4 in order to learn how to select a line.
- **Sequence:** generates a sequence of images. The number of images is defined in the Configuration window. If **Stage Shift** was selected from the **Setting** menu, the stage moves one step after each image of the sequence is acquired. Otherwise, the sequence corresponds to images scanned at the same stage position.

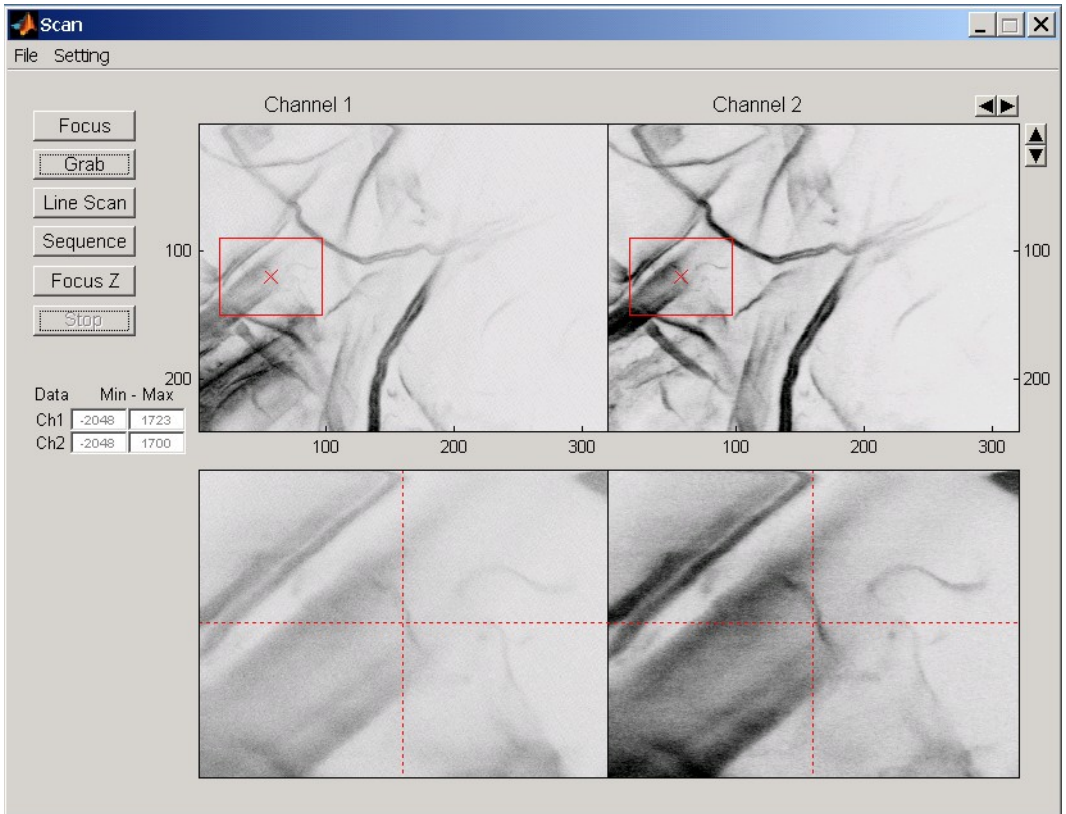

Figure 2: Extended main window.

- **Focus Z:** initiates the focusing process for the zoom-in area. This button is only enabled when **Focus Zoom-In** is selected from the **Setting** menu. The image is displayed in the extended part of the main window. If no zoom-in area was selected, the whole area will be scanned.
- **Stop:** stops the Focus and Focus Z processes, and abort any aother scanning fuction (Grab, Line Scan, or Sequence). Never close a window without stopping first the scanning process.

4.1.4 Other Functions

- **Area of Interest:** can be selected by clicking the mouse over the image displays, on the top part of the main window. After clicking once, a red cross enclosed by a red box will show the selected area, see Fig. 2. The size of red box is fixed by the zoom-in factor defined in the Configuration window (see Section 4.3). The zoom-in factor must be large than 100%. Clicking again will erase the selection area box.
- **Arrows:** located on the right top side of the image display, moves the selection area box in small steps. This function helps to make small adjustments in the position of the selection area box.

- **Data Display:** shows the minimum and maximum data value acquired from each channel. Since the acquisition board has 12 bits, the minimum and maximum possible values are -2048 and 2047. To update the reading, click with the mouse on the white area of the data display while you are focussing the microscope. Refer to Section 5 to learn how to use this information to set up the amplifiers.

4.2 Amplifier Window

When the **Amplifier** option is selected from the **File** menu, the Amplifier window is opened, see Fig. 3. This window allows the user to control and set up the SR570 current amplifiers. To learn more about the operation of this amplifier, refer to the equipment manual.

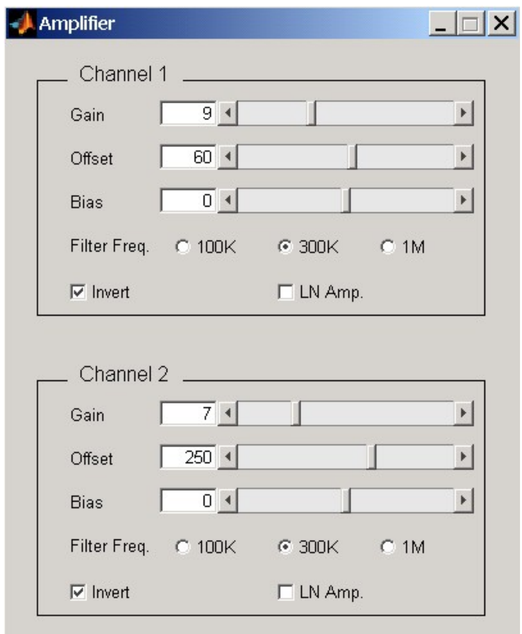

Figure 3: Amplifier window.

- **Gain:** sets the sensitivity of the amplifier in a logarithmic scale from 0 (1 pA/V) to 27 (1 mA/V).
- **Offset:** sets the DC current offset in an uncalibrated scale from -1000 to 1000; the offset can reach up to ±5mA.
- **Bias:** provides a variable voltage at the input in an uncalibrated scale from -5000 to 5000; the maximum bias is ±5V.
- **Filter Freq.:** selects the cutoff frequency of the low-pass filter.
- **Invert:** allows the user to invert the polarity of the amplifier output with respect to the input signal.

- **LN Amp.:** selects the low noise operation mode of the amplifier.

4.3 Configuration Window

When the **Configuration** option is selected from the **File** menu, the Configuration window is opened, see Fig. 4. In this window, the user defines several parameters related to the acquisition process. After changing the parameters, the **Apply** button must be pressed to load the parameters in the main window.

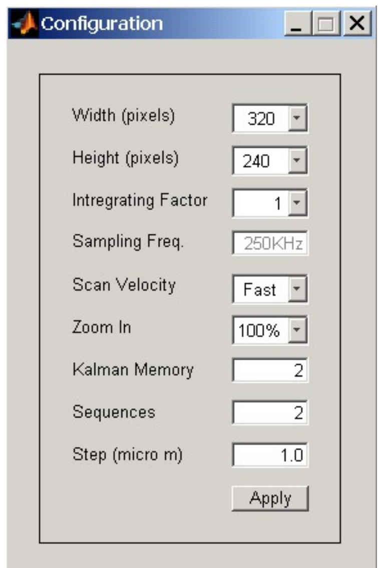

Figure 4: Configuration window.

- **Width:** defines the width of the scanned image in pixels for each channel.
- **Height:** defines the height of the scanned image in pixels for each channel.
- **Integrating Factor:** defines the amount of data samples added in one pixel. Selecting "1" means one data sample per pixel. When selecting larger values, the result is that the signal is (digitally) amplified and low-pass filtered.
- **Sampling Freq.:** shows the current sampling frequency. This value is calculated based on the former parameters, and can not be modified by the user.
- **Scan Velocity:** allows the user to select the scanning velocity: Fast (2.3ms/line) or Slow (4.6ms/line).
- **Zoom In:** defines the zoom in factor. The 100% value means no zoom is selected.
- **Kalman Memory:** defines the number of images to be averaged by the Kalman filter.

- **Sequences:** defines the number of images to be acquires for a sequence.
- **Step:** defines the step size in μm that the stage is moved between images. Positive values means upward and negative means downward.

4.4 Stage Window

When **Stage Shift** is selected from the **File** menu, the Stage window is opened, see Fig. 5. From this window, the user can control the position of the stage. Notice that when this window is opened, the joystick is disabled.

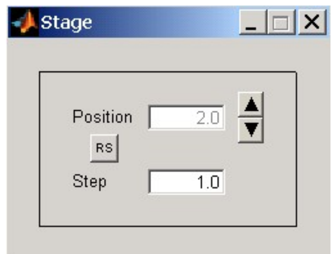

Figure 5: Stage window.

- **Step:** defines the step size in μm that the stage is moved between images. Positive values means upward and negative means downward. It is the same parameter described in Section 4.3.
- **RS:** resets the position of the stage. By clicking this button, the current stage position becames the reference or zero.
- **Position:** shows the position of the stage. The position can be updated by clicking in the white area of the position display, for instance, after a sequence had been acquire.
- **Arrows:** moves the stage upward or downward.

4.5 Grab, Line Scan, and Sequence Windows

After the **Grab**, **Line Scan** or **Sequence** button is pressed, a new window is opened to display the acquired image or sequence of images, see Fig. 6. The image can be saved as a TIF (8 or 16 bits) file selecting **Save as** from the **File** menu. The Sequence window has a scroll bar over the image that allows the user to see all the images.

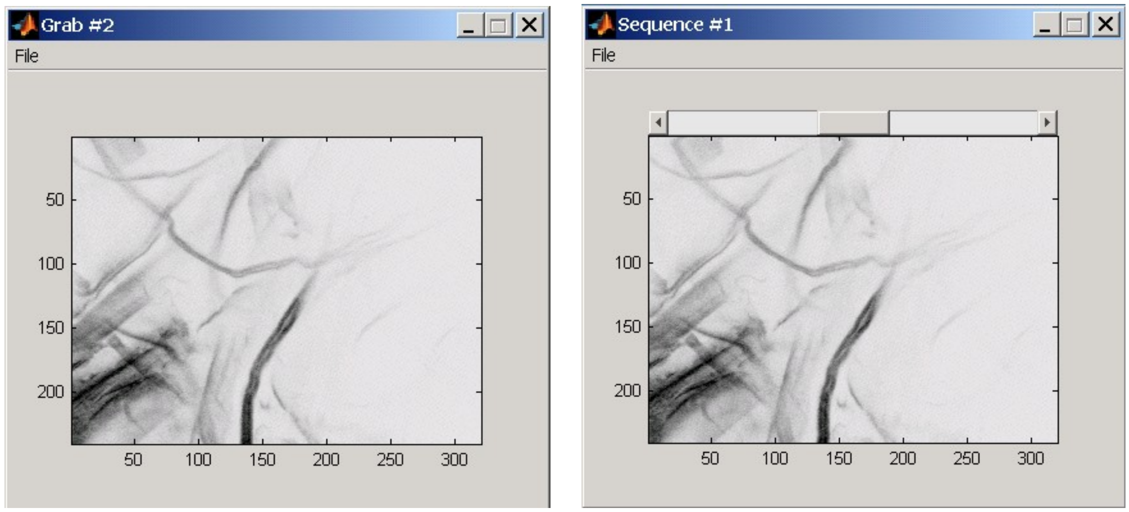

Figure 6: Grab, Line Scan, and Sequence Windows

5 Setting the Amplifier

This section explain how to set up the amplifiers using the **Data Display** to get good quality images.

Scan produce digital images by sampling the output signal of the photomultipliers with the acquisition board. The NI PCI-6110 has a 12bits analog-to-digital converter per channel. Thus, the signal coming from the confocal microscope can be quantified in 4096 levels with a dynamic range between -2048 and 2047. To get good quality images, the user must set up the amplifier to extend the signal over the whole dynamic range of the acquisition board. The following examples show different cases and how to use the **Data Display**.

1. When the signal covers the whole dynamic range of the acquisition board (see Fig. 7.a), the maximum and minimum value showed by the **Data Display** are slightly smaller than ±2047. Thus, the **Gain** and **Offset** parameters are properly set up.

2. When the signal amplitud is very low (see Fig. 7.b), the signal is being quantified by a small number of levels. Thus, the values showed in the **Data Display** are much smaller than 2047. The **Gain** should be increased until the numbers are close to ±2047.

3. When the signal amplitud is very large (see Fig. 7.c), it is said that the board is being saturated. Signal variations outside the dynamic range can not be measured. The values showed in the **Data Display** are 2047 and -2048. The **Gain** should be reduced until the numbers are close to ±2047.

4. When the signal is not centered in the dynamic range of the board (see Fig. 7.d), one of the values showed in the **Data Display** is close to zero and the other can be close to ±2047. The **Offset** should be changed until both the maximum and minimum are close to ±2047.

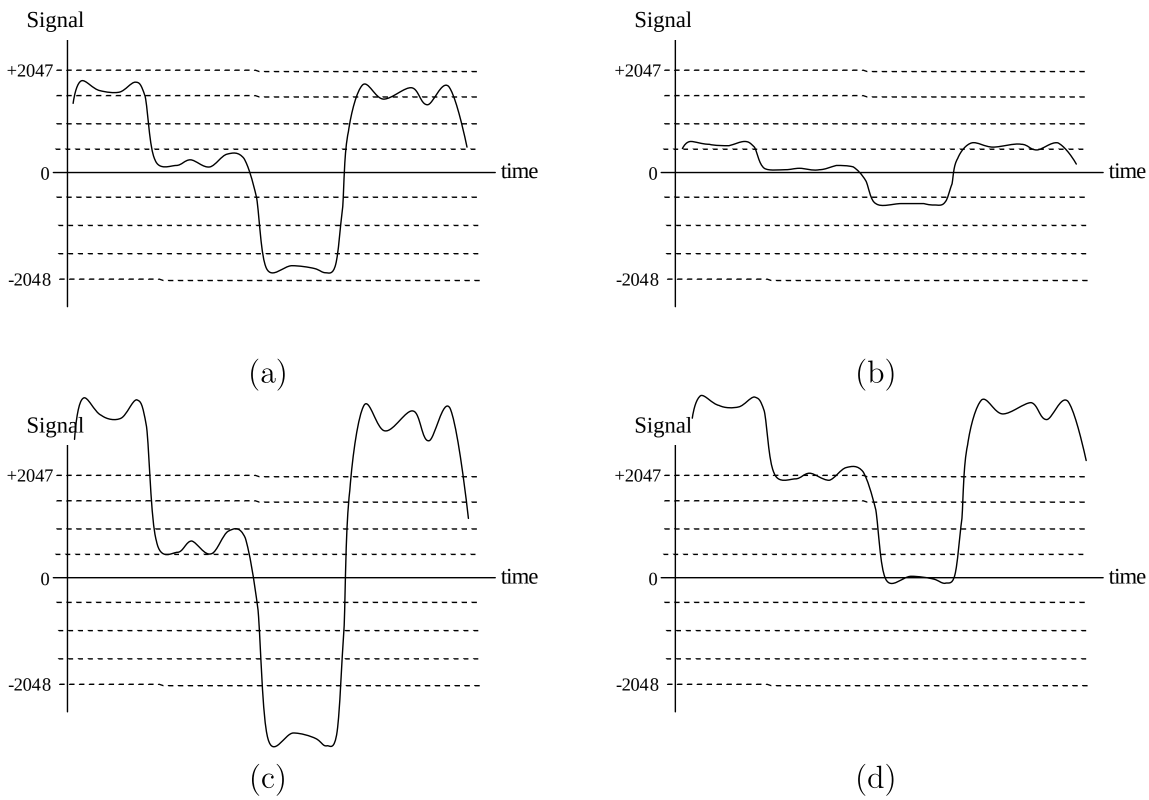

Figure 7: Setting up the amplifiers.

A Appendix: Software Structure

This section explains a few ideas about the *Scan* software. For a better understanding, the user should have some basic knowledge about programming and Matlab.

A.1 Graphical User Interface (GUI)

As it was discussed in Section 4, the software consists of a main GUI named Scan and several secondaries GUIs: Amplifier, Configuration, Stage, Grab, Scan Line and Sequence. These GUI were created and can be modified by the `guide` tool of Matlab. A GUI is defined by two files, one with extension `fig` and other with extension `m`, see Table 1. The `fig` file defines the graphical aspect of the GUI, and the `m` file defines the actions performed by the GUI: buttons, menus, mouse features, etc. For more information, refer to the "Graphical User Interface" Matlab manual.

A.2 Main Variables

The software parameters are saved as fields in a structure named `handles`. This structure with all the parameters is passed as an input argument to all the functions. The default

GUI	`fig` File	`m` File
Scan	`Scan.fig`	`Scan.m`
Amplifier	`AmplifierGUI.fig`	`AmplifierGUI.m`
Configuration	`ConfigurationGUI.fig`	`ConfigurationGUI.m`
Stage	`StageGUI.fig`	`StageGUI.m`
Grab	`ImageWindow.fig`	`ImageWindow.m`
Line Scan	`ImageWindow.fig`	`ImageWindow.m`
Sequence	`SequenceWindow.fig`	`SequenceWindow.m`

Table 1: GUI files.

values of `handles` are defined in the `start.m` file which is read when the software is launched.

Hardware

handles.BoardID: device number
handles.PAmp1: port COM for the Amplifier 1: 0=disable, 1=COM1, ..., 6=COM6
handles.PAmp2: port COM for the Amplifier 2: 0=disable, 1=COM1, ..., 6=COM6
handles.PStage: port COM for the Stepping Motor controling the stage: 0=disable, 1=COM1, ..., 6=COM6

Window Location (in pixels from the left lower corner of the screen)

handles.LocScaGUI: main window
handles.LocAmpGUI: amplifier setup window
handles.LocConGUI: configuration window
handles.LocStaGUI: stage control window
handles.LocDatGUI: data windows

Amplifier (X=1 for channel 1, X=2 for channel 2)

handles.SensitX: gain or Sensitivity control: 0 - 27
handles.OffsetX: current Offset: -1000 - 1000
handles.BiasX: voltage Bias: -5000 - 5000
handles.CutOffFreqX: filter Cutoff Frequency (KHz): 100, 300, 1000
handles.LNAmpX: filter Gain mode: 0=low noise, 1=high width band
handles.InvertX: invert the signal sense: 0=non-invert, 1=invert

Control and Display

handles.NumbPixels: number of pixels: 640, 320
handles.NumbLines: number of lines: 480, 240, 100, 50, 20
handles.NumbStrip: number of strip per image: 10, 5, 1
handles.NumbSequ: number of images scanned: ≥ 2
handles.BinF: bin factor: 4, 2, 1
handles.UseF: use factor or porcentage of used data (approximately 75%) - DO NOT CHANGE

handles.RaisT: raising part of the ramp (sec): 3.4e-3, 1.7e-3
handles.FallT: falling part of the ramp (sec): 0.6e-3, 0.3e-3
handles.InpFs: input sample frequency (Hz)
handles.OutpFs: output sample frequency (Hz)
handles.MaxValue: zoom-in factor (%): 800, 400, 200, 100
handles.OffSet: offset (pixels)
handles.Alpha: rotation angle (degrees), positive angles means counterclockwise rotation. DISABLE
handles.Kalman: Kalman filter: '1' is OFF, any number larger than 1 is ON
handles.KalmanMem: number of images averaged by the Kalman filter: ≥ 2
handles.MirrorDelay: delay of the mirrors (sec) - DO NOT CHANGE
handles.LS: line scan: '0' is OFF, '1' is ON
handles.Step: step size of the stage (micro meter): $.1 \leq$step≤ 1000
handles.Stage: movement of the Stage: '0' is OFF, '1' is ON

A.3 Main Functions

Beside the GUI functions, there are a few functions related to the scanning process. To learn about the the input and output arguments, type `help functionname` on the command line of Matlab.

- **SawSignal.m:** generates the output signal that controls the position of the X and Y mirrors.
- **CTRSignals.m:** controls the acquisition board counter to generate a pulse train synchronized with the X mirror. The signal is high (+5v) during the scanning and it is low (0v) during the fly-back. This signal can be used to turn off the laser during the fly-back.
- **NI.m:** defines parameters used by the board drivers. It is called by the CTRSignals function.
- **StripImageProc1C.m:** process the data coming from one channel of the acquisition board. This function removes the data acquiered along the fly-back and reshape the data in an array.
- **StripImageProc2C.m:** process the data coming from two channels of the acquisition board.
- **ScanProc.m:** starts the input and output porcess, acquires data to create the image using StripImageProcXC functions.

B Appendix: Color Map

To create an image, Matlab uses 256 color levels. Each data sample acquired by the board is quantified in one of these levels. The color for each level is defined in a color map table, which is a 256×3 array. The elements in the array are decimal numbers between 0 and 1. The columns of the table correspond to the color red, green, and blue, respectively. See Table 2 for some color examples.

Color	Row
White	1 1 1
Black	0 0 0
Red	1 0 0
Green	0 1 0
Blue	0 0 1

Table 2: Colors.

The user can create a customized color map by saving the array in a ASCII file. This file can be loaded by selecting **Load Color Map** from the **File** menu, as it was discussed in Section 4.1.2.

C Appendix: Wiring

This section explains how to interconnect all the pieces of hardware in the system. A list of requiered components is given at the end of the section.

C.1 Inside the Microscope

It is necessary to have access to the photomultiplier output. Open the microscope box, follow the coaxial cable from one of photomultiplier, and disconnect it from the amplifier board inside the microscope. Assemble a 5” long RG174/U coaxial cable with a SMB jack connector in one side and a BNC female connector in the other. Connect this cable to the photomultier output and replace the old BNC connector of the microscope. Repeat the procedure for the other photomultiplier. Then, close the microscope box.

C.2 Amplifiers and Motor Controller to PC

Connect the amplifier and the motor controller to the serial ports of the computer. For the amplifiers, use a DB9-DB25; for the motor controller, use a DB9-DB9.

C.3 Acquisition Board and Signal Conditioning Box

The NI PCI-6110 board has a 68 pin connector. To have access to the input and output signals, it is required a signal conditioning box. This box can be built by assembling a CB-68LPR connector block inside a CA-1000 case (the case comes with BNC female and DB9 male connectors); the NI PCI-6110 board can be connected to the signal conditioning box by a SH68-68-EP cable. All this products are from National Instruments. Table 3 gives a short description of the used pins; pin names are from the board manual.

#	Name	Description
68	ACH0+	Positive terminal of input channel 1; photomultiplier 1
34	ACH0-	Negative terminal of input channel 1
67	ACH0GND	Reference ground for input channel 1
33	ACH1+	Positive terminal of input channel 2; photomultiplier 2
66	ACH1-	Negative terminal of input channel 2
32	ACH1GND	Reference ground for input channel 2
22	DAC0OUT	Terminal of output channel 1; mirror X
55	AOGND	Reference ground for output channel 1
21	DAC1OUT	Terminal of output channel 2; mirror Y
54	AOGND	Reference ground for output channel 2
52	DIO0	Digital signal; internal trigger
17	DIO1	Digital signal; shutter
53	DGND	Digital ground for shutter signal
40	GPCTR1OUT	Counter 1; trigger counter 0
2	GPCTR0OUT	Counter 0; signal synchronized with mirror X
35	DGND	Digital ground for counter 0
11	PFI0	Triggers the acquisition process
5	PFI6	Triggers the output data process
41	PFI4	Triggers counter 1
3	PFI9	Triggers counter 0

Table 3: Signal descriptions for the board pins.

The pins depicted in Table 3 must be wired internally or to the connectors on the case. The connections should be made as follows:

- **Input Signals:** the input channels are connected to BNC connectors. For channel 1 connect pin 68 to the inner conductor of the BNC connector, and pin 34 to the outer conductor. Between pin 34 and 67, add a 10K-100K Ohm resistor. Similarly, connect the pins of channel 2 to another BNC connector.

- **Output Signals:** the output channel 1 and 2 (for the X and Y mirror signals) and the digital signal for the shutter are connected to a DB9 connector on the case, following the connections shown in Table 4.

- **Internal Trigger:** pin 52 outputs a digital signal which triggers the output and acquisition process when it changes from high (5v) to low (0v). Hence, connect pin 52 to pin 11, 5 and 41. Notice that an external trigger can be used replacing pin 52 by an external trigger source.
- **Counter Signal:** the output of counter 0 is a digital signal synchronized with the mirror X signal. To make it works, connect the output of counter 1 (pin 40) to the trigger input of counter 0 (pin 3).

C.4 Conditioning Box to Microscope

To control the microscope, three input signals are required: a) two analog signals, one for the mirror X and other for the mirror Y; b) one digital signal for the shutter (high=open, low=close). To have access to these signals, the microscope has a D-Sub 15pins male connector. These signals are generated by the NI PCI-6110 board, and they are available at the DB9 male connector of the signal conditioning box. To connect the signal conditioning box to the microscope, assemble multiconductor cable with a D-Sub 15P female connector in one side and a DB9 female connector in the other side. Follow the pin assignment given in Table 4.

D-Sub 15P	DB9	PCI-6110	Signal
1	1	22	Mirror X
9	6	55	Mirror X (ground)
2	2	21	Mirror Y
10	7	54	Mirror Y (ground)
15	3	17	Shutter
8	8	53	Shutter (ground)

Table 4: Pin assignment to connect the conditioning box to the microscope.

C.5 Microscope to Amplifiers

Connect the photomultiplier output of the microscope to the amplifiers using 50 Ohm coaxial cables with BNC male connectors.

C.6 Amplifiers to Conditioning Box

Connect the amplifiers to the signal conditioning box using 50 Ohm coaxial cables with BNC male connectors.

C.7 Complementary Hardware List

Qty	Description	Connection
2	SMB jack connector	Photomultiplier output
2	BNC female bulkhead connector	Photomultiplier output
2	5" long RG174/U coaxial, 50 Ohm	Photomultiplier output
2	Serial cable, DB9(female)-DB25(male)	Amplifier
1	Serial cable DB9-DB9	Motor controller
1	NI CB-68LPR connector block	Conditioning box
1	NI CA-1000 case	Conditioning box
2	BNC female connector panelette	Conditioning box
2	DB9 male connector panelette	Conditioning box
1	NI SH68-68-EP cable	Conditioning box to board
1	6' multiconductor cable	Conditioning box to microscope
1	D-Sub 15 pin female connector	Conditioning box to microscope
1	DB9 female connector	Conditioning box to microscope
2	Coaxial cable, 50 Ohm, BNC male connectors	Microscope to amplifier
2	Coaxial cable, 50 Ohm, BNC male connectors	Amplifier to condit. box

Table 5: List of hardware to interconnect the equipments.