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Differential Imaging Microscope System Acquisition Software Reference

Rev. A - September 2013

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Abstract

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NOMENCLATURE

AVT	Applied Vision Technologies
BCG	Brightness, Contrast, Gain
CCD	Charge-Coupled Device (light sensor)
CMOS	Complementary Metal-Oxide-Semiconductor active pixel sensor (light sensor)
DIC	Differential Interference Contrast
DLL	Dynamically Linked Library
DOE	Department of Energy
FFT	Fast Fourier Transform
GES	Gas Exposure System
GPIB	General Purpose Interface Bus (IEEE 488.2)
IMAQ	National Instruments Image Acquisition software
LED	Light Emitting Diode
MP	Megapixel
NI-MAX	National Instruments Measurement and Automation Explorer
PTFE	polytetrafluoroethylene (Teflon)
ROI	Region of Interest
RGB	Red, Green, Blue
SNL	Sandia National Laboratories
sub VI	sub-Virtual Instrument (subroutine)
USB	Universal Serial Bus
VI	Virtual Instrument

INTRODUCTION

Differential image processing is a technique through which subtle, time-dependent changes in an object being imaged can be readily observed. Using this technique, a series of high resolution images of a sample surface are taken over time, and then quantitatively compared to one another by subtraction. The resulting difference image consists only of the changes that took place in the time between the two images compared (see Figure 1).

Figure 1: Subtraction of an image taken at time t from an image taken at time t+Δt. Eliminate portions of the image which do not change. Resultant image nominally contains only those aspects which have changed.

Numerous benefits over traditional visual microscope inspection can be realized by this technique. A differential imaging system may be computer automated, resulting in both high positional accuracy and high rates of data acquisition compared to manual measurements. Quantitative analysis of image data for modeling corrosion behavior is possible, because a large number of site locations may be inspected at frequent time intervals through computer automation. To achieve these benefits, the imaging system must be highly automated and maintain a very high level of positional accuracy, requiring both excellent software control and mechanical design.

The differential microscope system described in this document meets these goals, but with greatly increased complexity compared to manual inspection systems. This increased complexity is due to many high precision hardware components - optical, mechanical, and electrical - and custom automation software. The hardware components are covered in the hardware overview section of this document and in greater detail in separate documents from the manufacturers of those components. The primary focus of this reference document is the custom automation software which has been developed by Sandia for this system, covering the operation and design of the differential image acquisition software.

SYSTEM HARDWARE OVERVIEW

Shown in Figure 2 is the differential microscope system hardware. This system is an automated microscope for high magnification image capture of a samples contained within an exposure chamber. A variety of sample geometries may be analyzed under continuous exposure to a corrosive gas atmosphere. K ey system components include an exposure chamber, positional control stages, microscope, illumination source, camera, and control instrumentation.

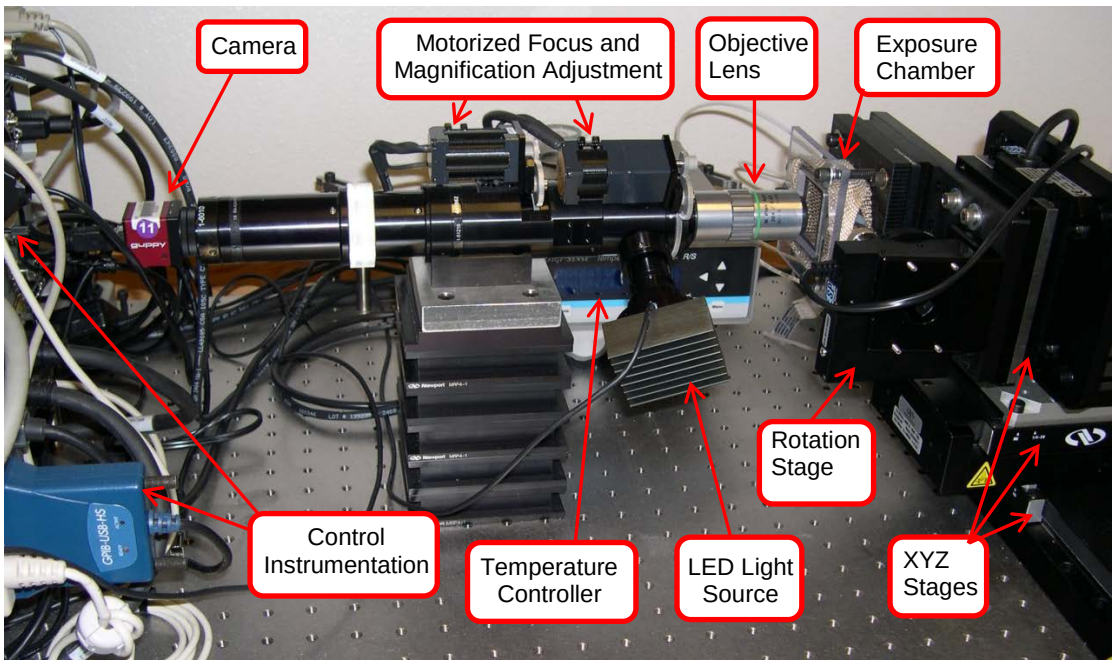


Figure 2: Differential microscope system hardware.

The heart of the differential imaging system is a 12x Navitar Ultrazoom microscope with 5-phase stepper motor control of focus and magnification. The components of the Navitar microscope are shown in Figure 3. The 5-phase stepper motors are powered by a controller with USB and RS232 communication interface for automated computer control by the LabView image acquisition program or via a windows program as per the *Navitar Motorized Systems Instruction Manual for Windows* and *Appendix B: Navitar Motor and Lamp Control Software Programs*. For both microscope stepper motor and light source controllers, only the RS232 connection supports 64-bit applications like LabView 64-bit. The USB connection is only usable with 32-bit programs, such as the Windows control software provided by Navitar, that run from x86 programs directory (see Appendix B). T he Other components of the microscope include an LED illumination source with power supply controller, Mitutoyo infinity corrected objectives, polarizing filter, analyzer, and extension tube. These components combined with an Applied Vision Technologies (AVT) Guppy F503C color CMOS camera with Firewire.B computer interface, comprise the optical system for capturing sample images.

To insure consistent sample illumination over time, a Navitar BrightLight II LED light source is used. This light source is powered by a control module with RS232/USB computer communications interface. Although the LED source provides excellent illumination consistency over time compared to bulb illuminators, illumination variation over the image area is significant. Image brightness is highest at the sample center, and image brightness correction may be needed when stitching adjacent images into composite images. Moreover, at low magnification image vignetting is present with this microscope system. The system operator should consider the best conditions for microscope configuration to produce a high quality image. Typically, a 250 micron wide image are has been used with the combination of a 20x objective, 1x adapter tube, 1200 steps motor position for magnification, and illumination power at 25-40%.

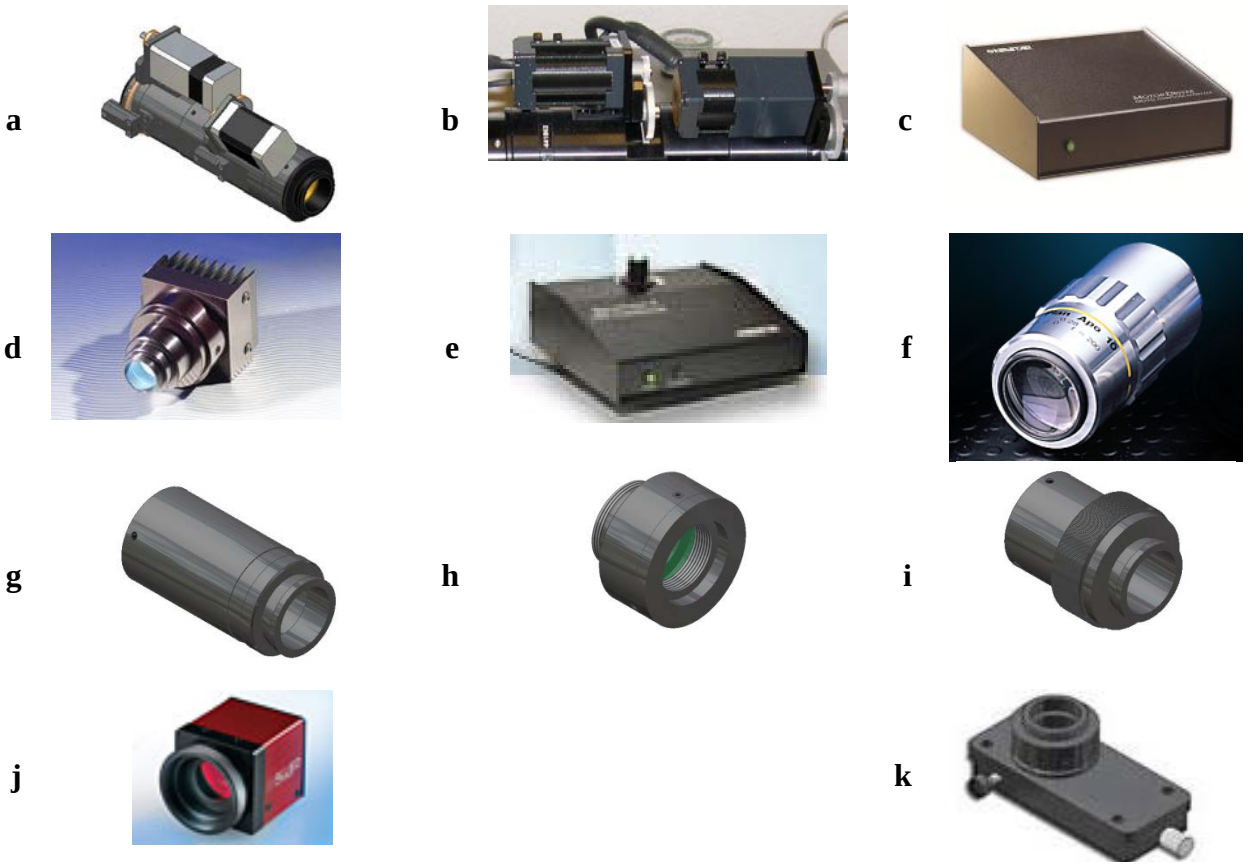


Figure 3: Microscope components: (a) 12x Ultrazoom microscope Tube, (b) 5-phase stepper motors, (c) stepper motor controller, (d) bright light illuminator, (e) illumination controller, (f) objective, (g) adapter tube, (h) polarizing filter, (i) analyzer, (j) camera, and (k) DIC module.

Mitutoyo infinity corrected objectives are available in 3 different magnifications of 10X, 20X, and 50X. These objectives have long working distances of 33mm for 10X, 20mm for 20X, and 13mm for 50X. The working distance is a key constraint when selecting sample type and magnification. Although the 50X objective offers the highest magnification, the 13mm focal length (working distance) is not usable with the current exposure chamber designs. Although either 10x or 20x objectives may be used, the 10x objective requires a 2x adapter tube for

optimum magnification range, resulting additional image distortion compared to a 1x adapter tube. The 20x is the optimum choice for most imaging applications.

A polarizing filter and analyzer are also available with the system to enhance surface contrast. The polarizing filter has a fixed angle and is inline mounted with the illumination module prior to light entering the microscope tube. The analyzer is comprised of a variable angle polarizing filter that is positioned between the microscope and adapter tube. When the analyzer is rotated the surface contrast is attenuated by constructive / destructive interference of the polarized light. To further enhance surface contrast, an optional differential interference module (DIC) attachment is available. The prism of the DIC module produces a Nomarski effect of the sample image. Although the result is improved visualization of the sample surface topology, image color is distorted, illumination non-uniformity increased, and ghost images of surface features may be present. A 10X objective works best with the DIC module reducing the illumination non-uniformity, because it is least sensitive to the DIC increased optical path length.

The final optical component of our system is the 5 MP (mega-pixel) Applied Vision Technology (AVT) Guppy F503C CMOS Firewire.B color camera. Under typical magnification conditions, with a field of view of 250 microns, each camera pixel corresponds to approximately 0.1 microns resolution. A CMOS camera was selected over CCD to reduce cost, because image capture speed for this system is approximately 2 frames per second and does not require the high speed / live motion capture that higher cost CCD cameras provide. The camera is also fully compatible with the IMAQ software drivers provided by National Instruments. Many camera parameters (such as color balance, gain, and shutter speed) are software configurable through National Instrument's MAX (Measurement and Automation Explorer) utility and LabView.

Shown in Figure 4 are the positional control hardware components of the differential microscope system. The motorized linear stages for YXZ positional control of the exposure chamber have a movement resolution of 0.1 microns. They include a Newport ILS-150CCHA for X-position control (sample horizontal position), a Newport ILS-50CCHA for focus control (sample distance to the microscope objective), and a Newport VP-25XA for Y-position control (sample vertical position). A Newport SR50cc rotation stage with 360 degrees of motion and a resolution angle of 0.01 degrees is available for curved pin samples. The XYZ linear stages are controlled with a 3-axis Newport Model ESP301 controller with x-position configured as stage 1, focus position configured as stage 2, and Y-position configured as stage 3. A second ESP301 controller is used for rotation stage control. Stage configuration, position, and homing may be controller through the user interface controls as per the *ESP301 User's Manual*. A joystick controller is also available for XYZ stage position control. Computer control of the two ESP301 units is though a GPIB to USB interface.

Positional stability of the sample is critical when acquiring images with the differential image microscope system over time. Because our image width is typically 250 microns, a 25 micron drift in position in the horizontal direction results in 10% of the differential image area not being usable (10% of the pre image area will not be seen in the post image). Figure 5 shows measurement data taken over 500 hours of continuous operation with $\pm$ 6 microns of position variation over a number of different sample site locations.

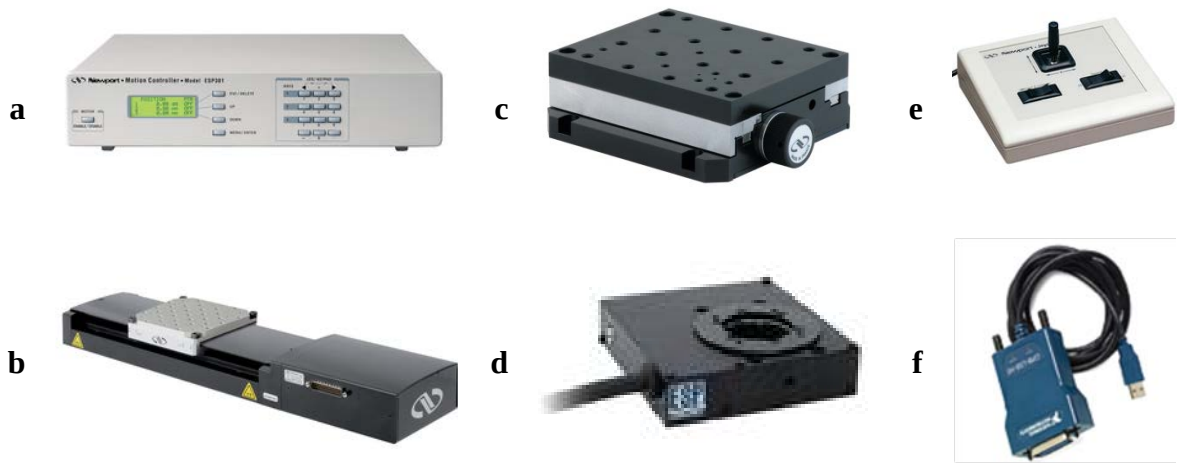


Figure 4: Positional control components include (a) two Newport ESP301 3-axis controllers, (b) Newport ILS-150CCHA and ILS-50CCHA linear stages, (c) Newport VP-25XA compact linear stage, (d) Newport SR50cc rotation stage, (e) digital joystick and (f) National Instruments GPIB-USB controller.

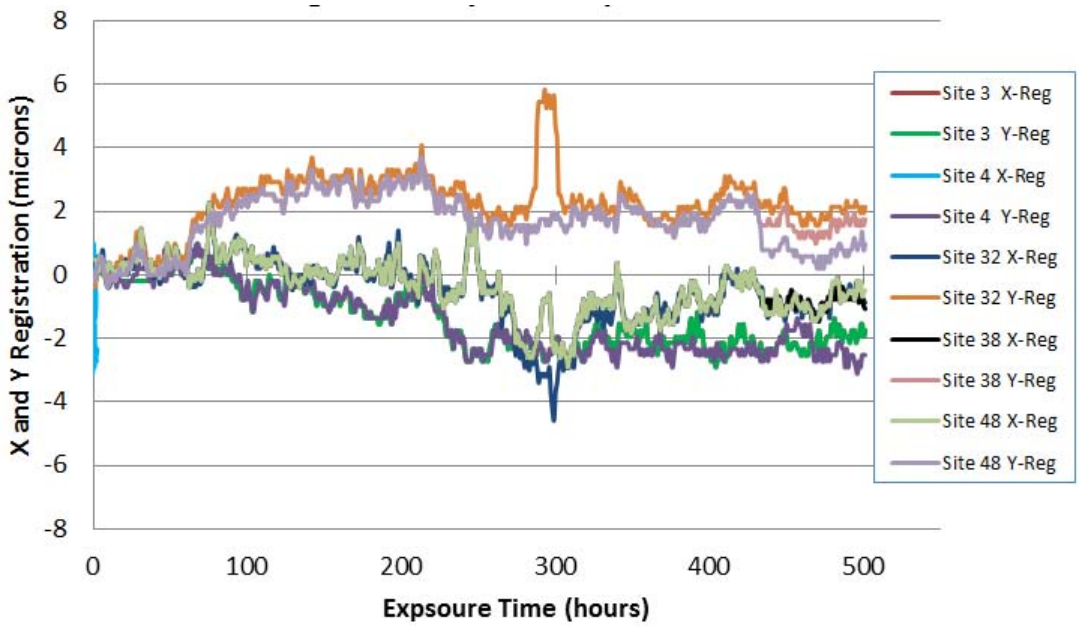


Figure 5: Rotated pin sample X-Y image registration (microns) versus time.

The final set of hardware components are the exposure chamber and gas exposure system. Present exposure chamber configurations allow either 2 cm x 2 cm flat coupons or cylindrical pin samples to be tested. Additional exposure chambers can be designed, or existing chambers modified, to accommodate other specimen geometries. Figure 6 shows components and a close up view of samples for the chamber configurations.

Flat coupon samples are held by 4 clips that hold the 4 edges of a square sample flat on mounting block. For flat coupon chamber configuration, a round quartz viewport separates the exposure

chamber / sample from the microscope objective which must maintain approximately 2 cm working distance from the sample surface for sharp focus with the 20x objective.

Curve pin samples are mounted in a groove in the rotation shaft. The shaft is held by the rotation stage using setscrews and an o-ring sealed FEP compression fitting on the exposure chamber. For curved pin chamber configuration, a square quartz window is used to separate the exposure chamber interior from the surrounding environment. Spacing between the microscope objective and sample is approximately 2 cm working distance with the 20x objective.

The exposure chamber is designed to maintain a corrosive gas atmosphere supplied by the gas exposure system (GES – Figure 7a). The GES supplies a mixture of gasses that match a Battelle Class II environment in the exposure chamber via PTFE tubing that attaches to a gas inlet compression fitting at the side of the chamber. A second compression fitting is for an outlet line plumbed to an exhaust vent. A liquid trap (a glass flask shown in Figure 7b) is present on the inlet line to capture condensation prior to reaching the exposure chamber. Chamber temperature is controlled to 35 °C with heat tape powered by a Digi-Sense temperature controller and monitored by a K-type thermocouple. Additional information on the GES system can be found in OP1892002, *Operation Procedure for the Gas Exposure Systems*, and OP1830036, *Procedure of the Facility for Atmospheric Corrosion Testing* (FACT).

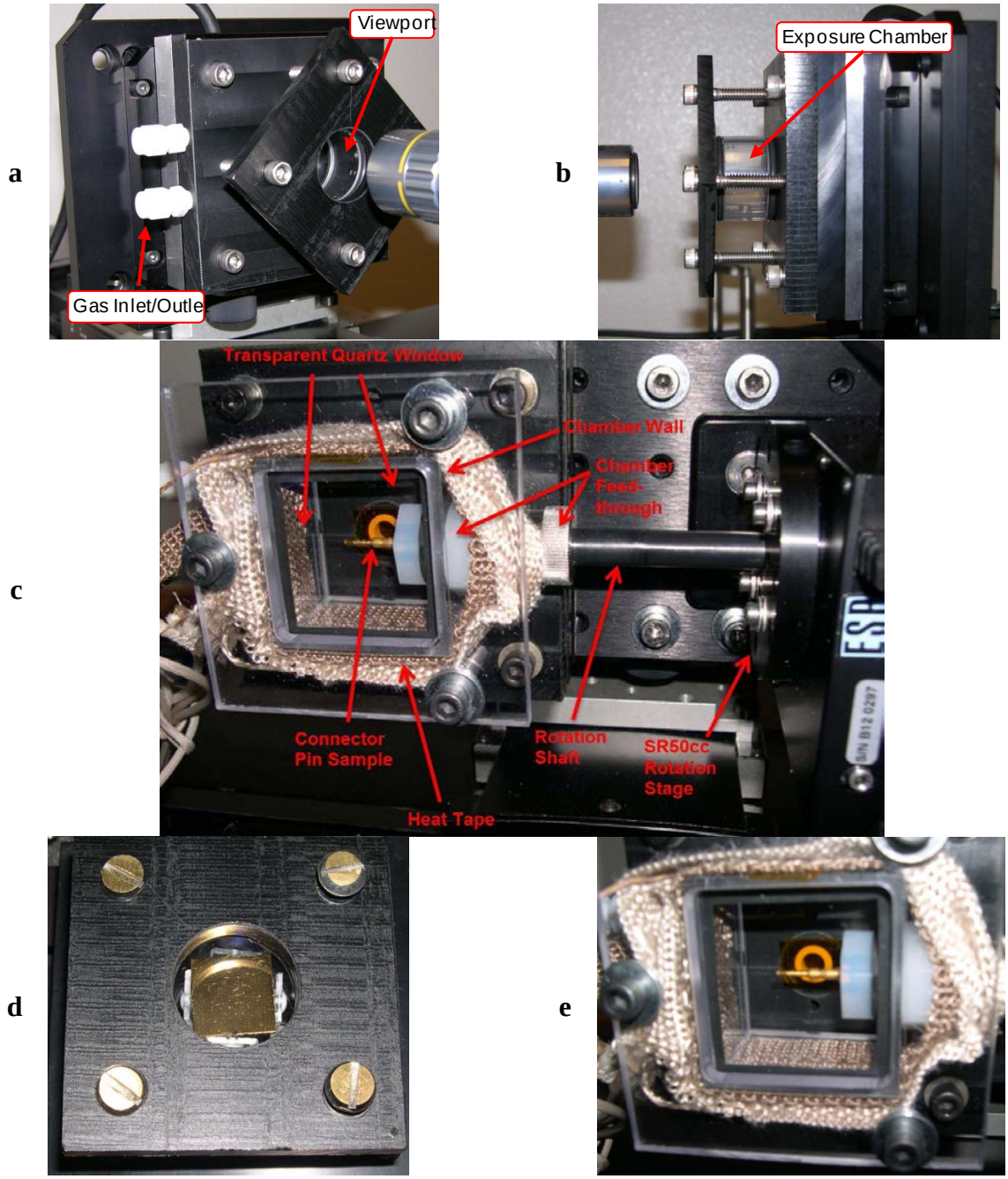
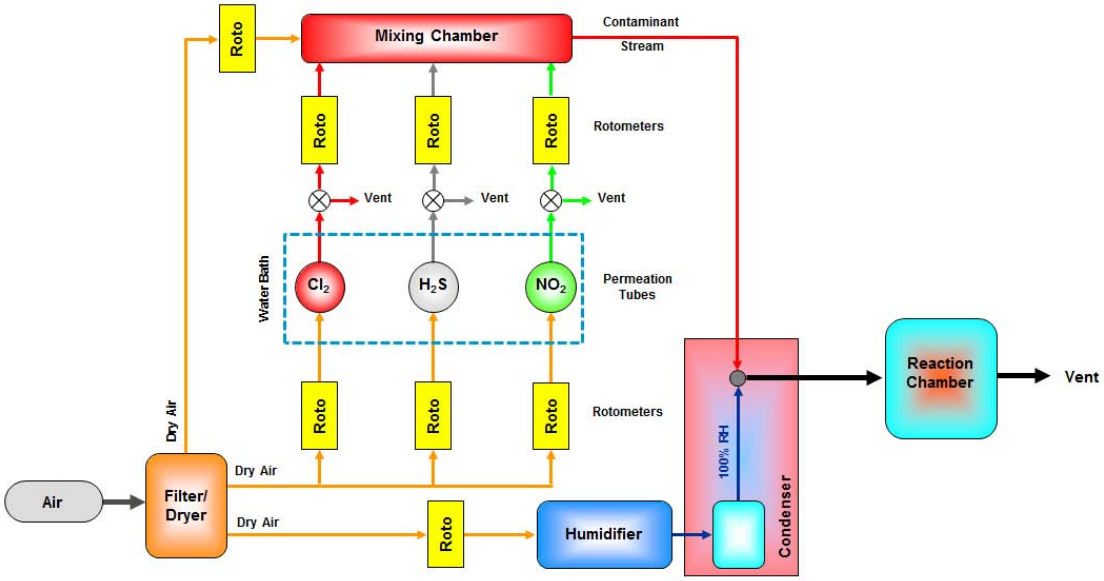


Figure 6: Sample holder and chamber for different test configurations. Environmental exposure chamber configuration for flat coupon (a, b) which allows for the flow through of a contaminant gas stream and optical monitoring of the sample via a viewport (a). In addition, the side of the chamber (b) can be wrapped with heat tape to allow for temperature control of the environment. Exposure chamber design for curved pin samples showing a rotation stage and shaft for 360o sample viewing angle (c). Flat coupon (d) and curved pin (e) mounted in chamber.

a



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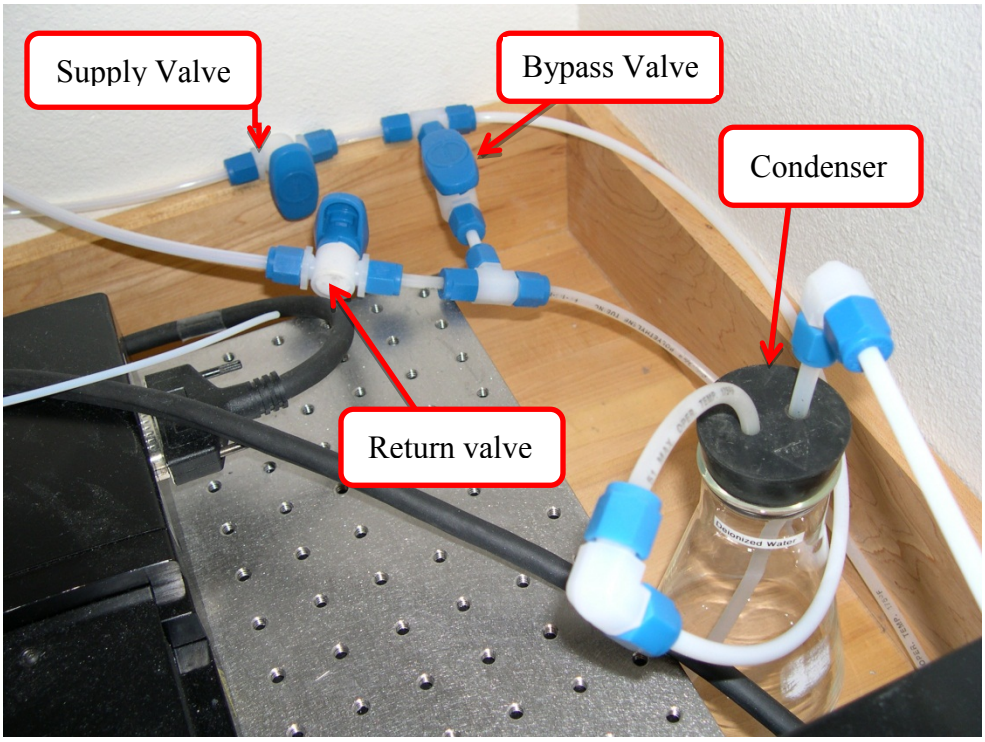


Figure 7: Gas exposure system components: (a) environmental control via a traditional mixed flowing gas system and (b) supply (inlet) / return (outlet) / bypass valves and liquid trap**.**

SOFTWARE DESIGN

The image acquisition software program was written using National Instrument's LabView graphical programming language and Vision Development Module. LabView is a rapid programming development environment designed specifically for data acquisition, and the Vision Development Module is an add-on module for image capture and processing. The software versions used are both 2012 and 64-bit. A 64-bit version of the software was selected to allow greater than 4GB of available memory for the application, which is the limit of the 32-bit software versions. When performing complex image processing on large arrays of images, such as image stitching, 10 GB or more of memory is required for proper system operation.

LabView programs are designated as VIs or Virtual Instruments and are divided into two key components: the front panel and the block diagram. The front panel contains numeric, text, button, graph, and image controls and indicators that comprise the user interface during program execution. The block diagram contains the program logic and structure. A VI may contain sub VIs which are subroutines called by the parent VI. The sub VIs provide modularity to the program, breaking common tasks into smaller blocks of program code.

The top level or parent VI for the LabView program is shown in Figure 8. The program contains 4 parallel loops that execute as semi-independent state machines. Each loop is dedicated to a specific operation. The program is complex with many states within each loop and a large number of user events linked to front panel controls that are registered with the Windows operating system (see Table 1). This design is a parallel loop event driven state machine architecture. Within a loop, each state is an atomic block of code that must be completed before a new state is started. When a loop is idle, an event listener is active that waits for user input by mouse or keyboard commands. After such a command, the loop will execute a sequence of one or more states before returning to idle. At the program start and finish, each state machine loop executes an initialization state and stop / termination state. The initialization state configures instruments and initializes controls/variables. Termination releases handles to instruments and frees system memory that has been used for references, queues, and images.

The image acquisition and positional control loop is the core of the program and handles both camera image acquisition and XYZ / rotation stage position. The loop contains an event handler for reading and responding to user inputs from controls on the front panel and uses the event driven state machine design pattern. The acquisition loop event handler is shared with the illumination control loop via a queued message handler. This message handler allows the main loop to control and monitor lamp illumination during auto-run operation. Automated system operation is executed within this loop, and this loop includes the program logic for autofocusing using the Newport focus stage. This loop also controls program termination with the three other loops having listeners to determine when this master loop ends, triggering the end of execution of all loops.

Navitar microscope magnification/focus and illumination control are handled through two individual loops. Because control of the Navitar microscope is outside of the image acquisition and positional control loop, the stepper motors and lamp module can be operated either within the LabView image acquisition program or operated by separate software programs from Windows or a standalone LabView program. This capability was necessary when Navitar could not provide 64-bit DLL software drivers for their controllers, and their 32-bit DLL files

were found incompatible with 64-bit LabView. Eventually, the DLL files were rewritten by Sandia for 64-bit using RS232 control and are now compatible with 64-bit LabView, but this capability to operate the microscope motors and lamp outside of the acquisition program was retained. Moreover, keeping control of these components separate does not impact performance. The image acquisition program relies on the Newport ILS-50CCHA focus stage with superior 0.1 microns step resolution (along with an optical encoder) for automated focus control, rather than the Navitar focus stepper motor. Illumination and magnification remain constant during auto-run operation, with illumination being queried periodically through a messaging queue.

The fourth loop of the program controls image analysis and advanced features like image subtraction, defect size measurement, image stitching, and site mapping. Many of these operations can be executed while the system is operating in auto mode acquiring image data. This allows a user to analyze the data being taken without interrupting new data from being acquired. Like the image acquisition loop, this loop is an event driven state machine design. This loop also contains many ROI (region of interest) tools for aiding the user in image processing.

Although the top level VI is highly complex, the complete program is considerably larger. Figure 9 shows the large number of sub VIs (subroutines), custom controls and dynamic link libraries used by the top level VI. The design is modular with many sub VIs being reused in different parts of the program.

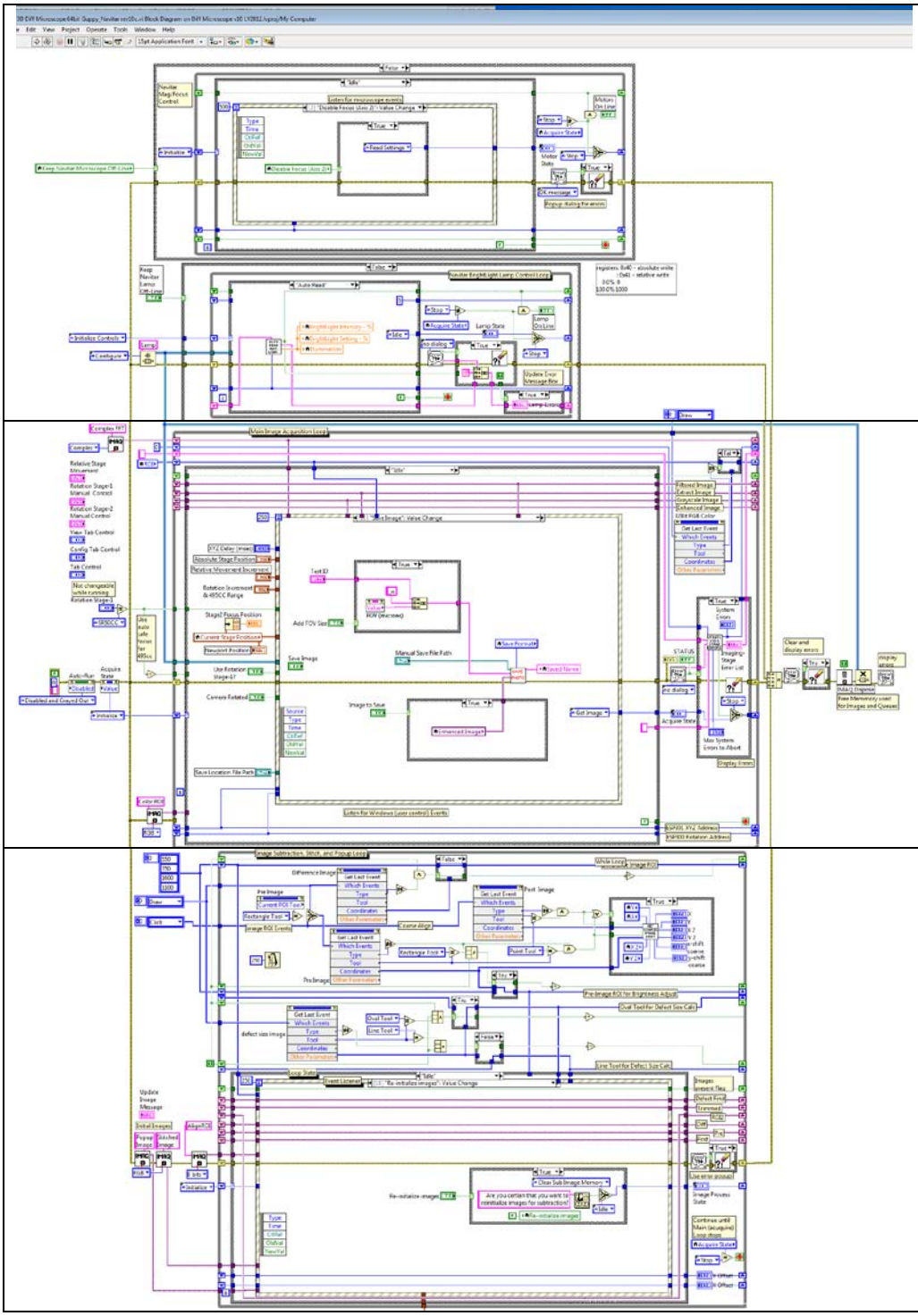


Figure 8: The LabView application program software design utilizes 4 parallel loops that run semi-independently. The image acquisition and sample position control loop is the main program loop.

Table 1: The states and events handled by the 4 parallel loops of the LabView program

Loop	User Events	States
Image Acquire & Stage move	51	Idle, Initialize, Newport AutoMove, AutoFocus, Get Image, Save Image, Disable Controls, Enable Controls, Stop
Image Analysis	19	Idle, Initialize, Configure, Auto Align, Update Images, Disable Controls, Enable Controls, Clear Sub Image Memory
Navitar Motor Control	14	Idle, Initialize, Configure, Reconnect Motors, Read Settings, Stop
Navitar Lamp Control	None (message queue)	Idle, Initialize Controls, Configure, Reconnect Light, Write, Read, Auto Read, Stop

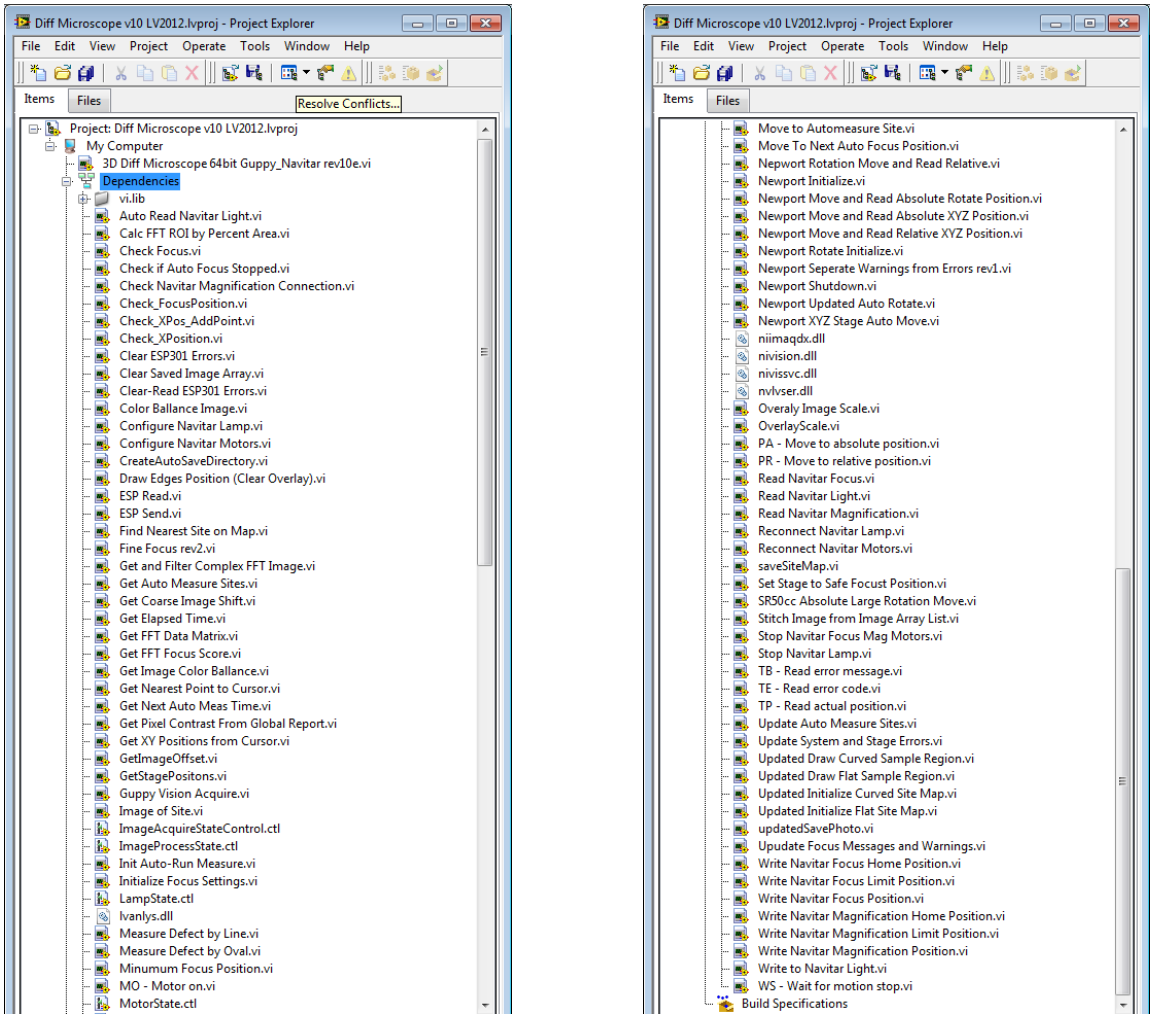


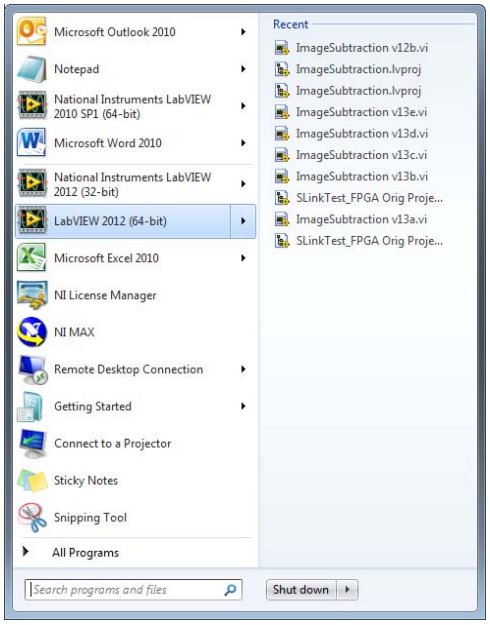
Figure 9: Underlying structure of the LabView application program with 87custom sub VIs, 4 custom controls, 5 dynamic link libraries (DLLs).

GETTING STARTED

The first step to running the image acquisition program is to open up LabView 2012. Left click the Windows 'Start' button and navigate to the LabView 2012 (64-bit) shortcut (Figure 10a) or navigate to 'National Instruments->LabView 2012 (64-bit)->LabView (64-bit)'. This will start the LabView software environment (Figure 10b).

After starting LabView, open the image acquisition program project by navigating to and left clicking on ' C:\Diff Microscope\LV2012 Diff Microscope Guppy 3D\Diff Microscope v10 LV2012.lvproj' (Figure 10c). T his will open the project associated with image acquisition program. Left click on the VI '3D Diff Microscope 64-bit Guppy_Navitar10e.vi' to open up the LabView top level VI (Figure 10d). The LabView image acquisition program will then open up showing the user interface front panel (Figure 10e).

a



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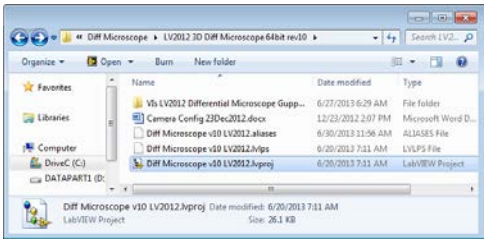

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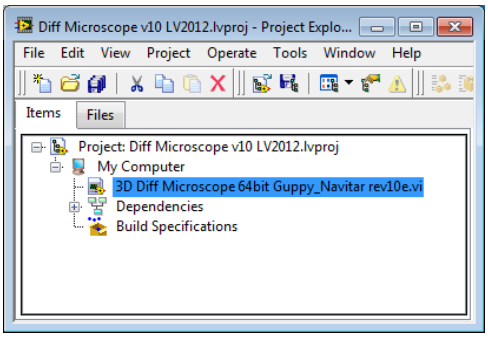

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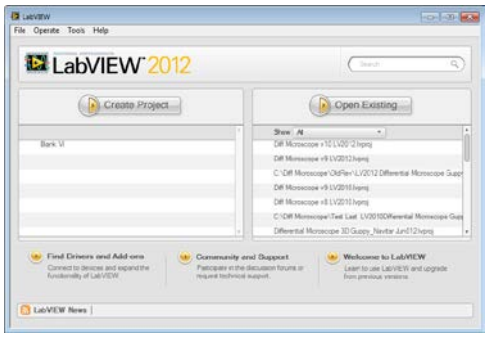

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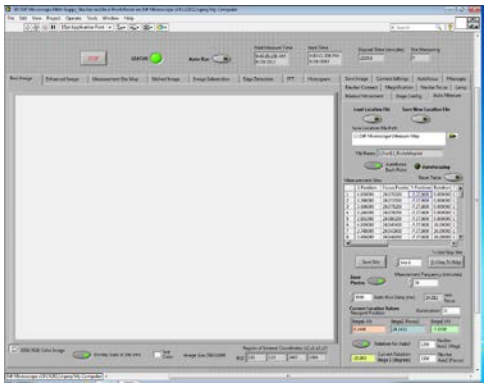


Figure 10: Windows screen views seen when starting LabView and the image acquisition software program: (a) navigation from the start button to the LabView 2012 64-bit program, (b) The LabView 2012 program start window, (c) location of the LabView program using Windows Explorer, (D) project view of the image acquisition program, and (e) user interface / front panel of the program

SOFTWARE OPERATION OVERVIEW

The user interface for the image acquisition software is divided into two sets of tabbed windows. Figure 11 shows the front panel display, which is the user interface during operation. The leftmost set of tabs is dedicated to data display and analysis, showing acquired images, wafer maps, analyzed images, and image focusing tools. The rightmost set of tabs is dedicated to user controls. It includes stage position controls, stage configuration, auto-measure site coordinates, camera settings, image save options, microscope operation, and error/warning messages. In addition to the two blocks of tab controls, a line of controls is present at the top of the program front panel. The 'Stop' button allows the user to terminate the program. The 'Status' button displays green if no error is currently being reported and red when an error occurs. The 'Auto Run' button is used to switch between auto mode operation completely under computer control when lit green and manual operation when non-illuminated. The next measure time and start time indicators show timestamps for auto run operation, recording when the next set of measurements are due and when the first set of measurements were initiated. Elapsed time and site measuring displays show the current progress of auto run operation.

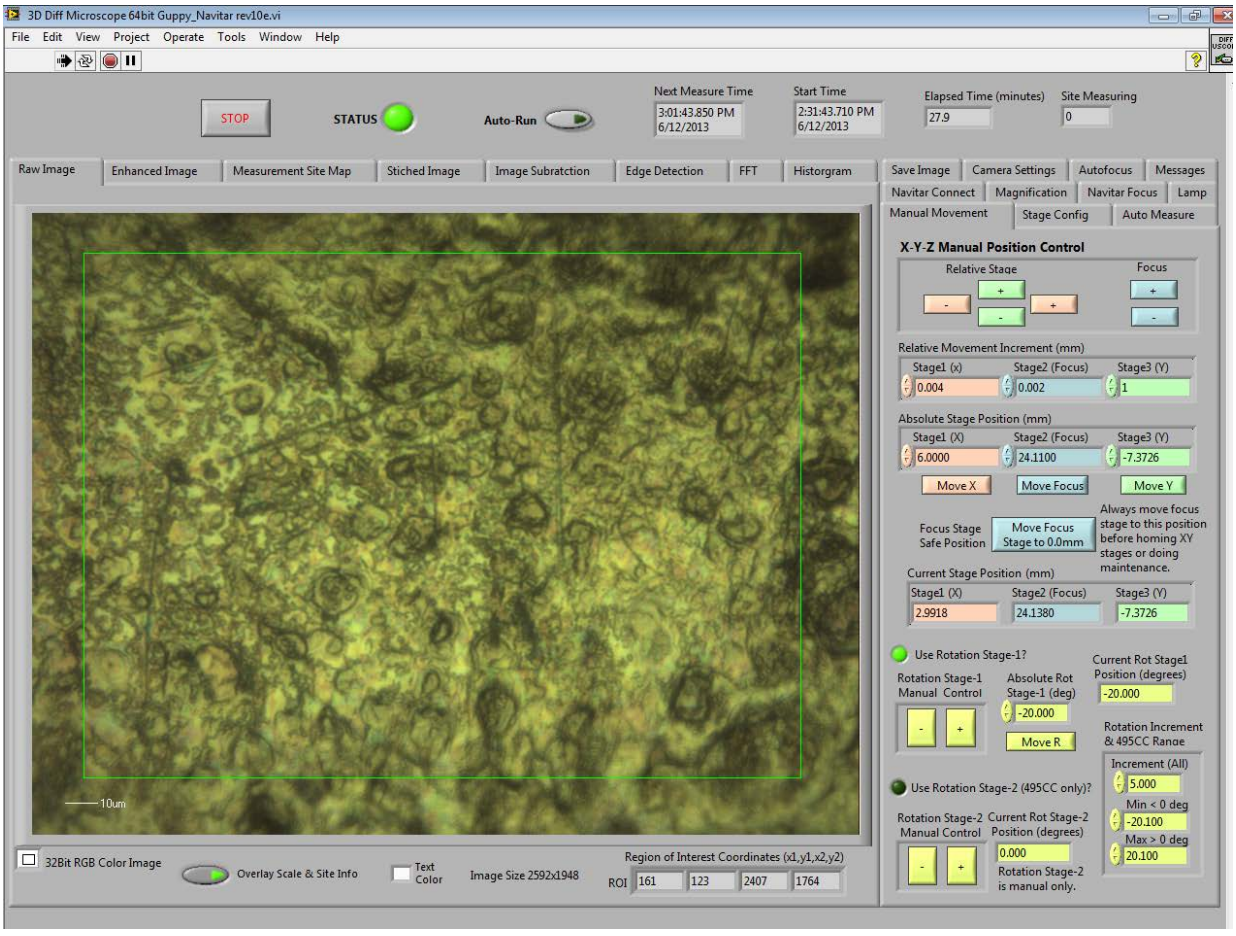


Figure 11: Image acquisition program front panel. The left set of tabs show image and measurement data. The right set of tabs are operation controls and messages.

MICROSCOPE IMAGE CAPTURE AND CONTROLS

Figure 12 shows the image data view tab panels. The 'Raw Image' tab displays the image as viewed by the Guppy camera without modification. This tab has a minimal number of options that include the an overlay display of image information that can be modified to different text colors and also includes a rectangular region of interest (ROI) tool that selects the portion of the image used for autofocus. 'Enhanced Image' is the second tab display and includes controls for altering image brightness, contrast, alpha and RGB color balance. Although the enhanced image may show additional details not seen with the raw image, this option is not recommend for acquiring images for differential image analysis. Enhanced images are software altered and may not be representative of the true sample surface. The third tab panel has an overall map view of measurement sites for auto-measure or auto-run operation. The saved sites from the "Auto Measure" measurement sites table may be uploaded to the map display by pressing the 'Load Site from Auto Measure' button. These sites may then be navigated either by selecting the site number or using a cursor control. When using the map control, the user may select between map displays for flat coupon or curved pin samples using a toggle switch at the top of the panel. A sample origin / center, sample size, and the sample perimeter coordinates should be entered by the user. These locations are determined by manually navigating over the sample surface using the manual stage controls (to be discussed later in Figure 15). Previously saved images of sites taken in auto run mode may be displayed by pressing the 'View Saved Image' button. *Appendix G: Measurement Site Mapping* covers map view operation and options in greater detail.

The control tabs panels for acquiring images are displayed in Figure 13. These image acquire tabs include image save, camera configuration, and autofocus. The save image panel includes a 'test ID' text control for entering the file name for images saved. A toggle button allows the user to either save the raw image (exactly as acquired by the Guppy camera) or an enhanced (modified image with the options selected from the enhanced image tab). During auto run operation, the site number and a timestamp will be add to the end of the file name Two directory path navigation controls are provided to allow the user to select where image files will be saved. The manual save file path control is used when the 'Manual Save Image' button is pressed on this same control panel. The auto save file path is only used in auto mode. There is an additional option to auto create (and save to) sub-directories with the current date under the selected auto save directory. Near the top of the panel is a control to note if the camera has been rotated 90^{o}, changing horizontal and vertical and should be disabled during typical operation. At the bottom of the save image tab are controls for determining the image horizontal size in microns and displaying the measured size in the image names. To determine image size for a microscope magnification, the user navigates (using the manual stage controls) to a selected feature and positions it the left side of the image window and presses 'Set' left. The user then moves that same feature to the right side of the image window and presses 'Set' right. Image width is determined when the 'Calc' button is pressed. The second tab includes options for configuring the Guppy AVT camera. These options should not be altered from their defaults values without first consulting the equipment owner. These brightness and gain values override the camera setting for image luminance set in the IMAQ setting of MAX (see Appendix A). The white balance settings for blue and red can be used to increase or decrease the intensity of these colors relative to green. These settings and the color correction options at the bottom of this tab are controls/displays specific to color correction measurement that area described in detail in

Appendix D: Camera Color Calibration. The remaining control panel is autofocus control. It will be discussed in autofocus section of this document.

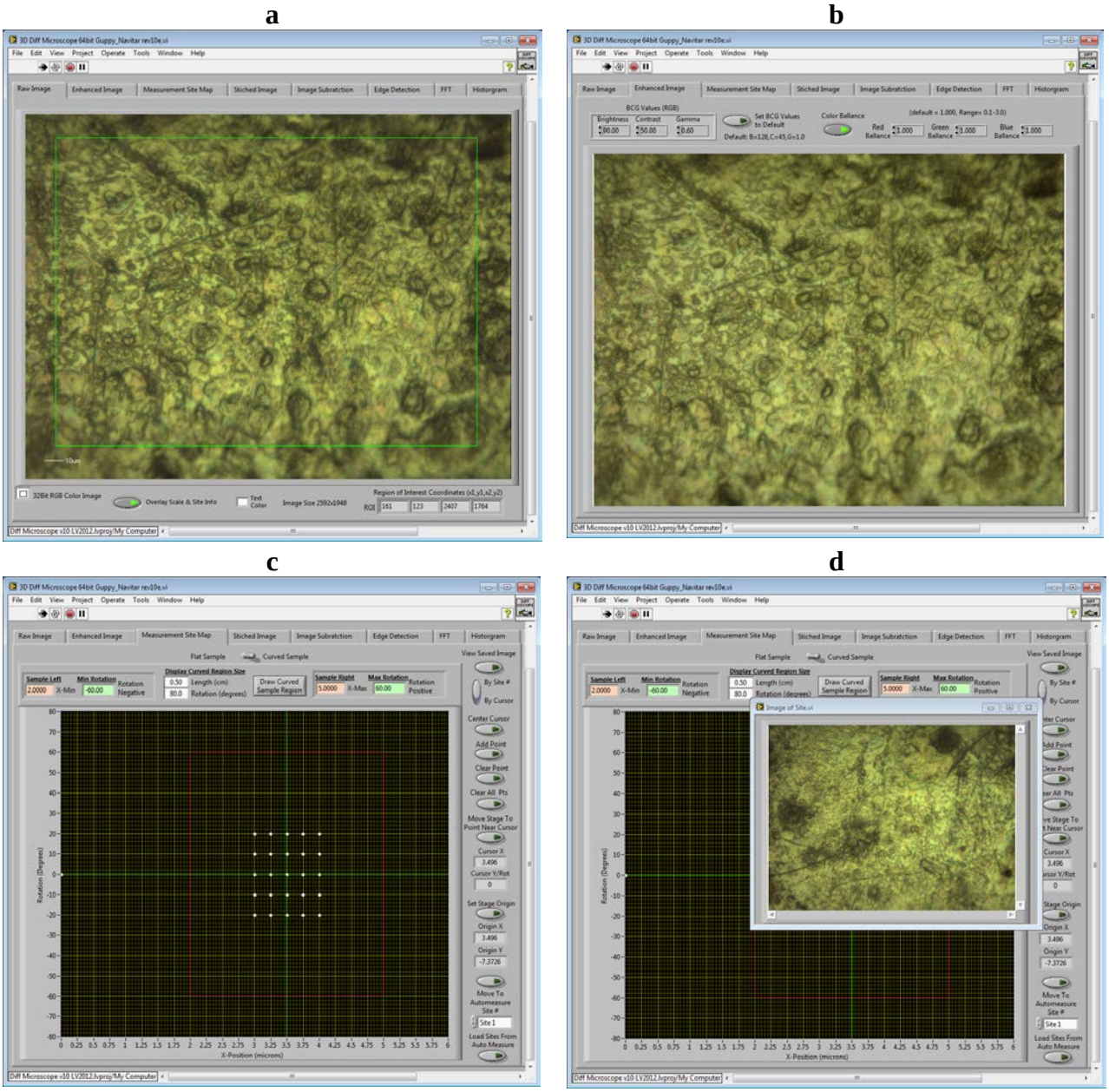


Figure 12: Image data view display tabs (a) raw images as acquired by the Guppy camera, (b) enhanced images that have been software modified with BCG and RGB adjustments, (c) site map display, and (d) image popup display of selected image site.

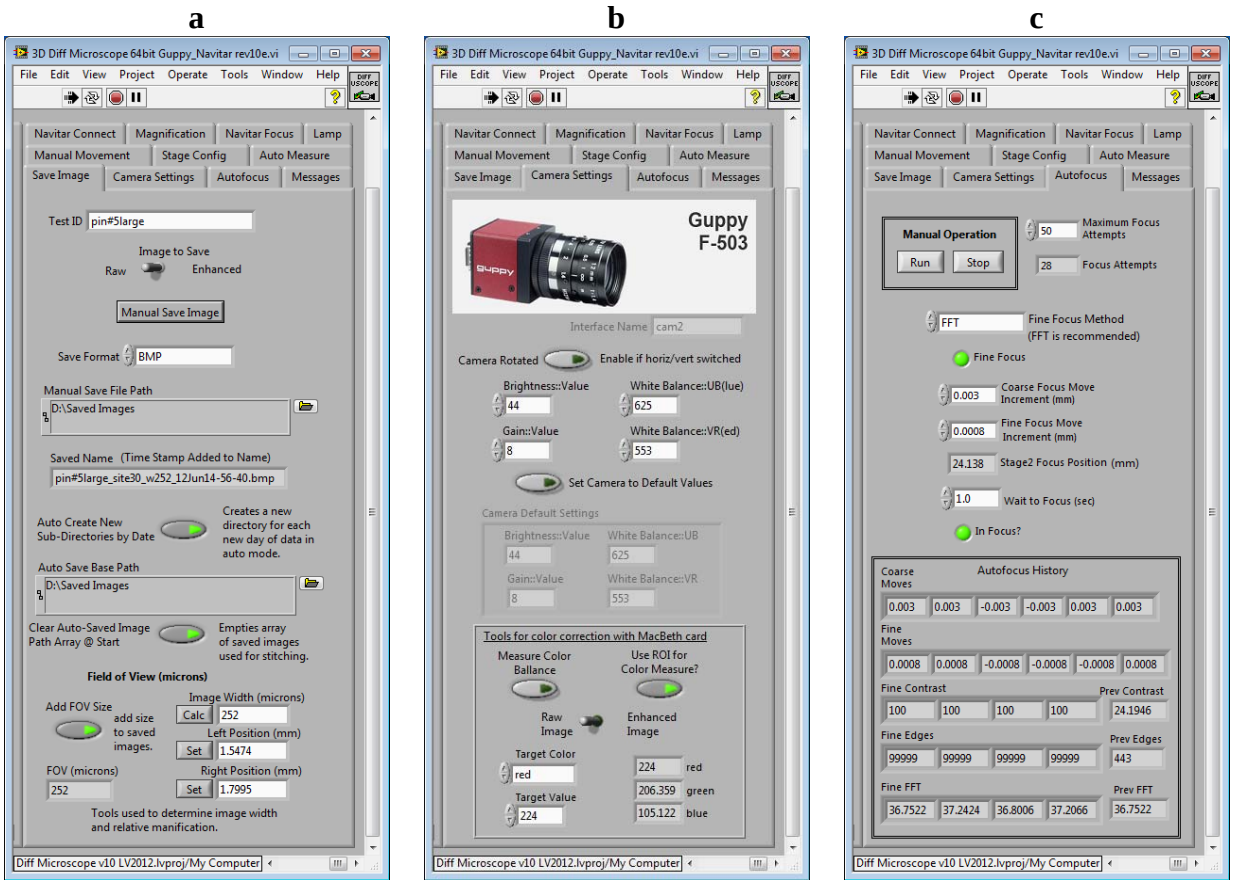


Figure 13: Control tabs panels for (a) image save options, (b) camera setting configuration, and (c) autofocus**.**

Control tabs for the Navitar microscope and illumination source are shown in Figure 14. Integrated microscope and lamp control by the program may be toggled on/off prior to program start, providing the option to control of the microscope / illuminator using Navitar provided software by selecting the 'Off-Line' option. C urrently only RS232 serial control is available with 64-bit LabView and the COM ports must be configured for the stepper motor controller (COM1) and lamp controller (COM2). T he stepper motor controller is responsible for microscope magnification and focus. Both of these are set by a number of steps that are typically 3300 for focus and 1200 for magnification. 1200 s teps magnification should correspond to an image width of approximately 250 m icrons, when using the Navitar microscope with 20x objective, 1x adapter tube, and 5MP Guppy camera. Although image focus may be controlled manually with the Navitar stepper motor controller, the precision of the Newport focus is superior, and only the Newport stage is controllable by autofocus. It is strongly recommended that the Navitar focus stepper motor be set to a fixed value (such as 3300) and left constant. When first starting the LabView software, the operator should run the stepper motors from home to max position a few times and verify stepper motors consistently go to a maximum position of 2123+/-10 steps for magnification and 6775+/-25 steps for focus as per *Appendix B: Navitar Microscope Manual Control Software*. The illuminator should be set to a brightness that produces a high contrast image at the selected magnification.

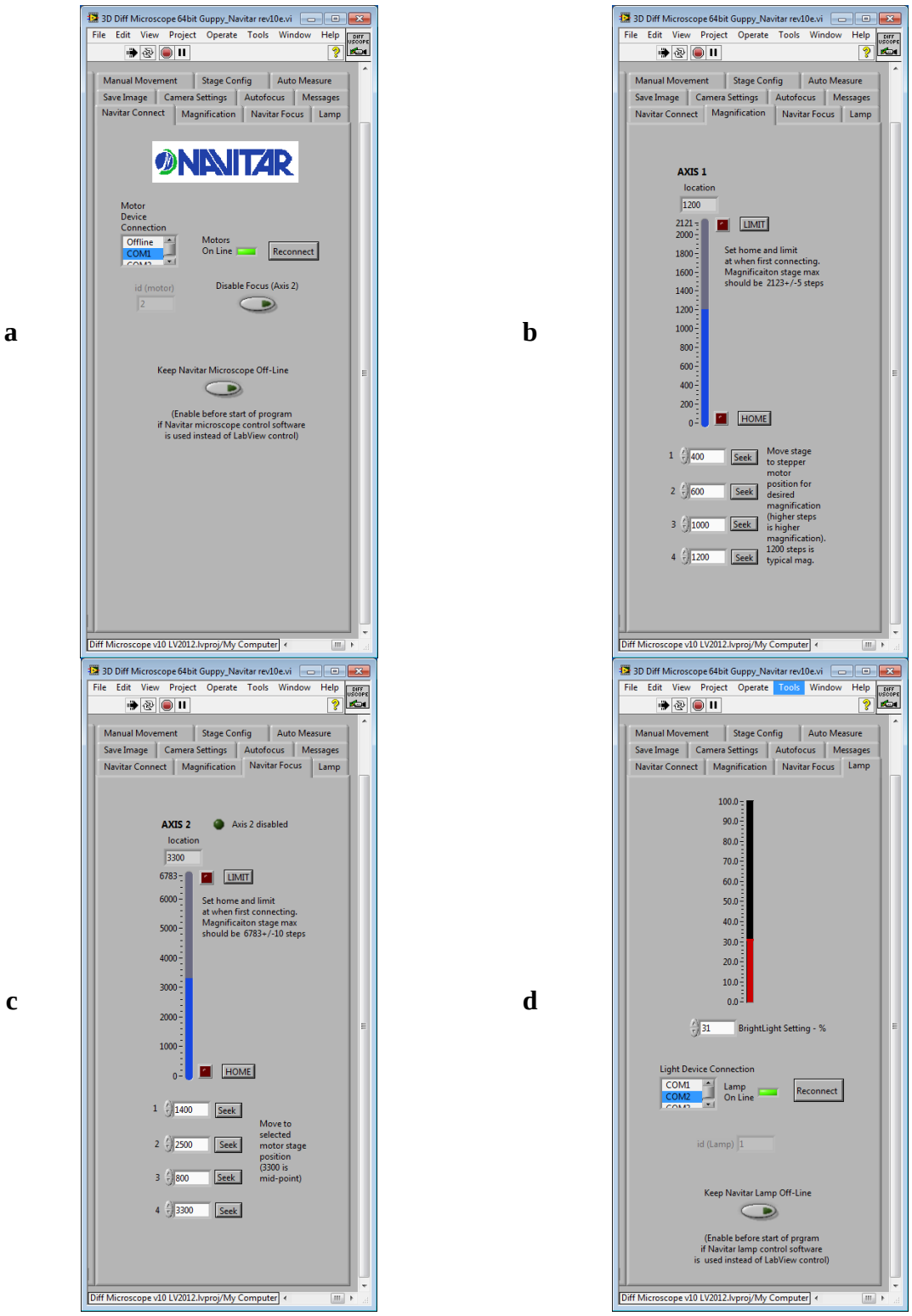


Figure 14: Control tab panels for Navitar microscope and lamp operation: (a) stepper motor configuration, (b) magnification stepper motor control, (c) focus stepper motor control, and (d) lamp illumination control.

Stage configuration and movement are controlled with the tab panels displayed in Figure 15. Figure 15a shows the configuration options for the Newport stages and the ESP301 controller. The Newport linear XYZ stage positions are controlled by the Newport ESP301 configured at GPIB address 1. Stage 1 corresponds to sample horizontal position and is the longest ILS linear stage located at the bottom of the stack of XYZ stages. Stage 2 corresponds to sample focus position and is the shorter ILS stage immediately atop the horizontal stage. Stage 3 corresponds to vertical position with a VP25X compact linear stage at the top of the stack of XYZ stages. Rotation stage(s) are controlled with the secondary ESP301 controller at GPIB address 2. Typically, only stage 1 is configured with this ESP301 controller, using a SR50cc rotation stage that controls curved pin rotation. Optionally, a second rotation stage (495cc) may be added for manually adjusting sample pitch angle. The stage configuration tab includes warning limits to alert the operator of potential collision with the microscope objective. The X (stage 1 / horizontal) and focus (stage 2) limits should be set by the operator to appropriate values if the sample chamber is reconfigured or the stages are repositioned on the optical mounting block. The remaining controls on the stage configuration tab are for stage delay time. The delay time is a wait time to allow the stage to reach position before the next stage command is sent. This feature avoids having multiple commands being queued to the controller and possible GPIB communication errors.

Figure 15: Control tab panels for (a) Newport stage configuration, (b) manual stage movement, and (c) auto measurement.

The ‘Manual Movement’ tab panel (Figure 15b) is used for controlling stage position when not in auto-run mode. The topmost set of controls are buttons to step the XYZ stages a selected relative distance either positive or negative direction. The absolute stage position controls move the stage to selected position, rather than relative distance. In the middle of the tab is a large blue button to move the focus stage to 0.0mm position, which is a safe location to be prior to homing the other XYZ stages. At the bottom of the tab panel are rotation stage controls.

Similar to the XYZ linear stage controls, the rotation stages may be moved either a selected relative distance or to an absolute stage position. The relative movement distance is set by the rotation increment in angle degrees. Rotation stages may be enabled (lit LED) or disabled (dark LED) in the software by pressing the “Use Rotation Stage” green LED button on this panel (Figure 15b). If a 495cc stage is used, max and min limits for stage position angle should also be set to avoid having collision between the exposure chamber and microscope objective. The SR50cc rotation stage is capable of 360 degrees of movement and ignores these min and max angle limits. When both the 495cc (as stage 2) and SR50ss (as stage 1) are enabled, they share the same rotation increment step size control for relative position movement but have separate absolute position controls and current position indicators. In this configuration, the 495cc stage will be set to a constant pitch angle to align the sample perpendicular to the microscope objective. After this pitch angle has been set, the 495cc stage should remain stationary.

The remaining movement tab control panel is for auto measurement or auto-run operation. Physically and operationally central to this tab panel is the measurement site table. Up to 100 sites may be saved to this table. The site table may be manually created by moving to each desired measurement site, select the site number for assignment, and pressing the ‘Save Site’ button. This operation will overwrite any previous information for that site. If a new sample is started, it is recommended that this table be erased by pressing the ‘Reset Table’ button. After a table of desired measurement sites is created, the table should be saved to a tab delimited text file when pressing the ‘Save New Location File’ button. This file will be saved at the location specified in the file path control with the name of the current timestamp + ‘siteMap.txt’. The saved file can also be uploaded at a later time using ‘Load Location File’ button. The site measurement table specifies XYZ and rotation positions for each site. Also included is a ‘?’ column that selects if the site will be used for auto-run operation. By selecting the site number from the pull-down control and pressing the ‘?=Use/Skip Site’ button, the site will be toggled between being used (value of 1) or ignored (value of 0) during auto-run operation. Additional controls on this tab panel include enabling ‘Autofocus Each Point’, ‘Save Photos’, ‘Measurement Frequency’, ‘Auto-run Delay’ and ‘Rotation for Auto?’. These options when selected either enable/disable that feature or a time period for measurement frequency and auto-run delay. Measurement frequency specifies how often a new set of measurements is taken for each site in measurement site table. Auto-run delay sets a delay time GPIB commands, to avoid GPIB communication errors with the ESP301 controllers. Near the bottom of the panel are a set of stage position and microscope position/illumination indicators that display these values during auto-run operation. Additional information on auto-run operation is provided in *Auto-Run Operation* and in *System Operation: Automated Measurement* sections.

The final block of tab controls are for messages from system errors, warnings, or system loop states. Figure 16 shows the message tabs with four state indicators displayed: (a) acquire state (main loop), (b) image process state, (c) lamp state, and (d) motor state. The indicators display

the current state of each of the software program loops (Figure 8). On the errors tab panel, the operator can specify a program abort limit for maximum system errors allowed. System errors are either stage movement or camera IMAQ acquisition failures. There is also a lamp error display for communication errors with the Navitar lamp controller. Because only the lamp intensity is monitored and not controller during auto-run, lamp errors are not counted as system errors that might force an automated abort of auto-run operation. Focus warnings (failure to determine the optimum focus point position in the specified maximum attempts) and stage warning messages (stage 0 errors or no error messages) are not included as system errors. Errors due to Navitar motor control are also excluded from system errors because the Navitar microscope is controlled in manual mode and left constant during auto-run operation. Navitar microscope (focus / magnification) errors appear as pop-up dialog boxes, because they only occur during manual operation.

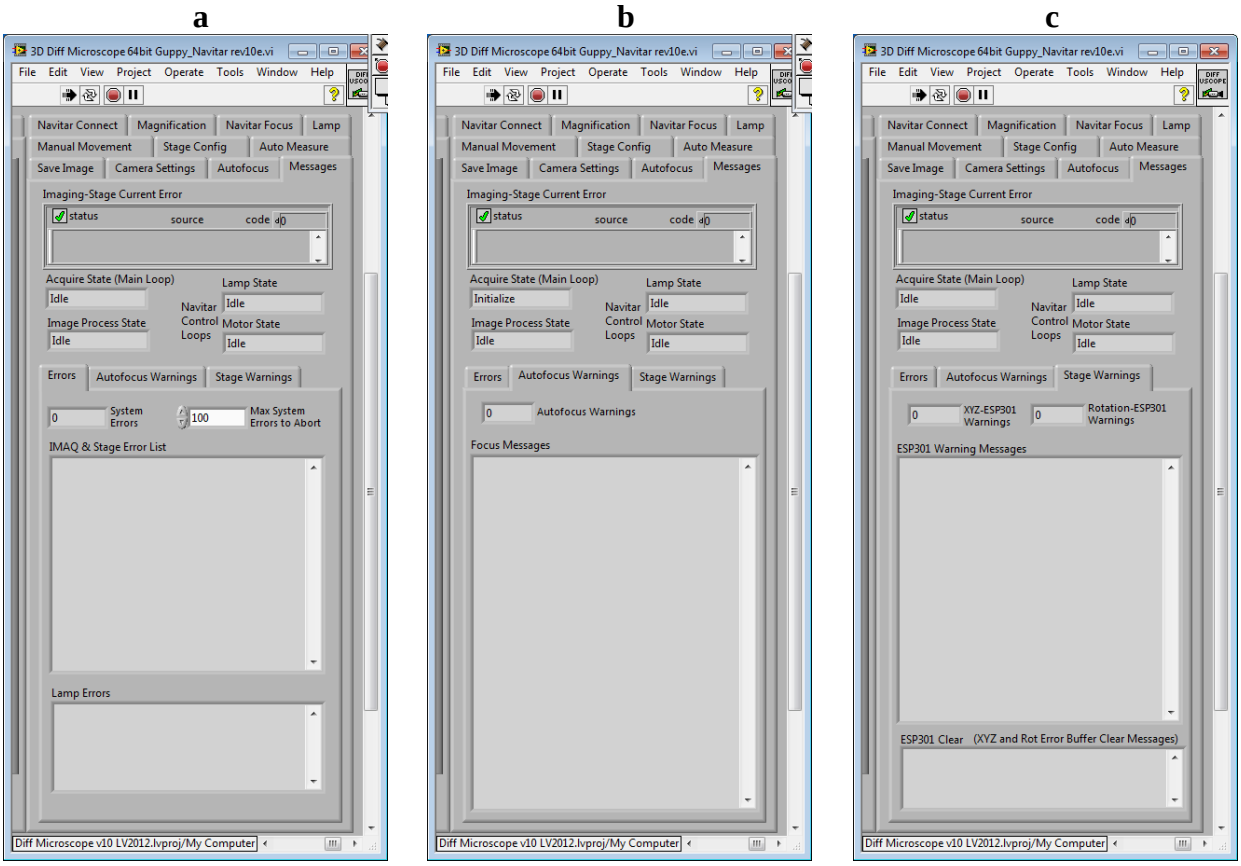


Figure 16: Control tab panels showing messages for (a) system and lamp errors, (b) autofocus warnings, and (c) stage warnings.

AUTOFOCUS

Acquisition of sharp images is critical for our system. Automated focus algorithms were developed in the software to insure images acquired were in focus. The background source materials for developing auto focus algorithms with LabView and Vision Development Module include the Image Processing with LabView and IMAQ Vision by Thomas Klinger and the NI Vision Concepts Manual.

Figure 17 shows the last group of data tab panels for image focusing by edge detection, FFT, and histogram methods. Also shown is the autofocus control tab, which is operated in unison with the focus data panels. The focus method (edge, FFT, or histogram/contrast), stage step increment(s) / wait time, and maximum number of focus attempts are global options set from the autofocus control tab. Autofocus is executed with the options selected when 'Start' is pressed and is terminated when an optimum focus position is found. Autofocus may also be terminated when the 'Stop' button is pressed or if a maximum number of focus attempts (the sum of coarse and fine focus attempts) has been reached. Indicator displays show the number of focus attempts currently executed, fine focus execution (coarse focus has completed), in focus (optimum focus point found), and a number of focus history array displays showing values for previous focus attempts. The focus algorithm uses a two-step method of first obtaining coarse focus to move quickly from a blurred to moderately sharp image followed by fine focus to optimize sharpness. The coarse focus method is not changeable and exclusively uses the histogram or contrast method. This method is best for taking a blurred image and achieving improved but not optimum sharpness quickly. Pixel contrast across the entire image or a selected ROI is maximized, as maximum % standard variation / mean. During coarse focus that Newport focus stage is moved in step sizes set on the autofocus tab. Coarse focus is determined when the focus stage movement oscillates between two distances of the stage step size. Once coarse focus is achieved, the fine focus indicator is illuminated. One of three fine focus methods is executed with a user selectable step size that is typically ¼ the coarse focus step size.

The recommended fine focus method is FFT (Fast Fourier Transform), but histogram and edge detection are also available. The histogram fine focus method is similar to the coarse focus method but with smaller step size. Obtaining sharp image focus by this method is difficult and will likely result in image to image sharpness variation. The edge detection method measures the number of lines and similar abrupt features within the image. An edge is a large contrast change between adjacent pixels as specified by the edge options. Edge options include looking only at rising (dark->bright), falling (bright->dark), or both edges. Additional options include the kernel size (number of adjacent pixels to analyze in a single edge), edge width (in pixels), minimum edge strength (pixel intensity difference), interpolation type, and data processing method. Please refer to Vision Concepts Manual for additional information on edge detection configuration and options. Although edge detected can successfully achieve fine focus, it has difficulty with low contrast surfaces that result from heavy surface corrosion.

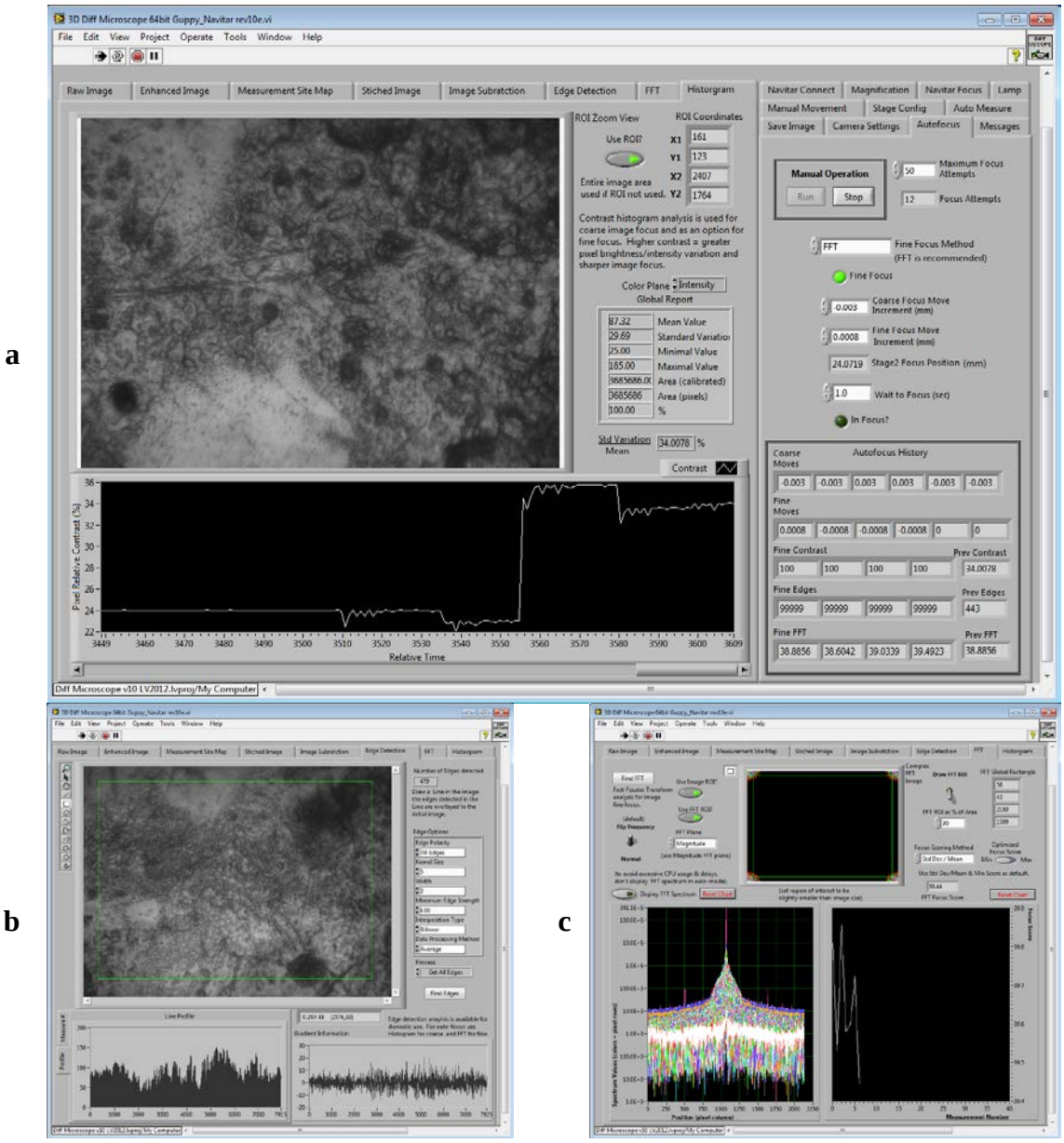


Figure 17: Autofocus tab panels showing the three focus methods available (a) histogram (image pixel contrast), (b) edge detection, and (c) FFT.

FFT is the preferred fine focus method. Image pixel intensities over row and column positions are converted to magnitude (real) and phase (imaginary) values that correspond to a frequency distribution. The magnitude values correspond to the image brightness variation and can be filtered by a variety of methods to remove values that occur in higher or lower frequency. Figure 18 shows various options for obtaining sharp FFT focus. Two approaches have been demonstrated. Both methods filter out high frequency values from the population to maximize sensitivity to gray colored pixels. The first method (Figure 18a-c) uses the standard (normal) FFT calculation method, applies a frequency filter to remove a specified percentage of high pass frequencies, and optimizes for maximum calculated root mean square magnitude values. The second FFT focus method (Figure 18d-e) uses the reciprocal of magnitude values (flipped

frequency), applies a ROI filter (remove 10% of values = use 90% of area) to the FFT image that removes high frequencies, and optimizes for minimum standard deviation divided by mean of values. Although a less common method for FFT focusing than the first approach, this approach has been proven the most reliable and is recommended for sharp image focus with our system.

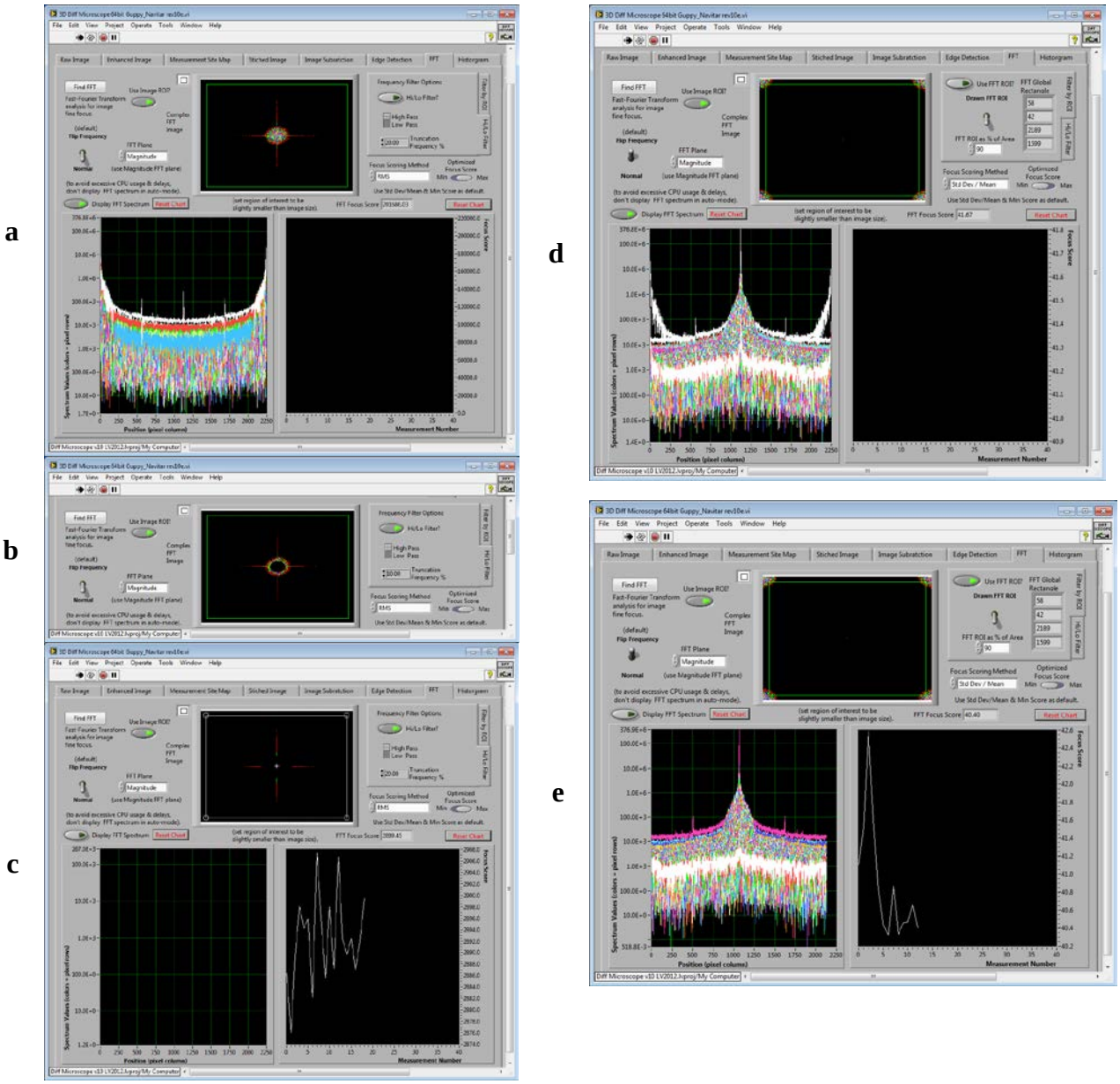


Figure 18: FFT autofocus: (a) normal FFT conversion with high pass filtering of (b) 10% and (c) 20%. Reciprocal or (d) flipped frequency FFT conversion with (e) ROI filter of image.

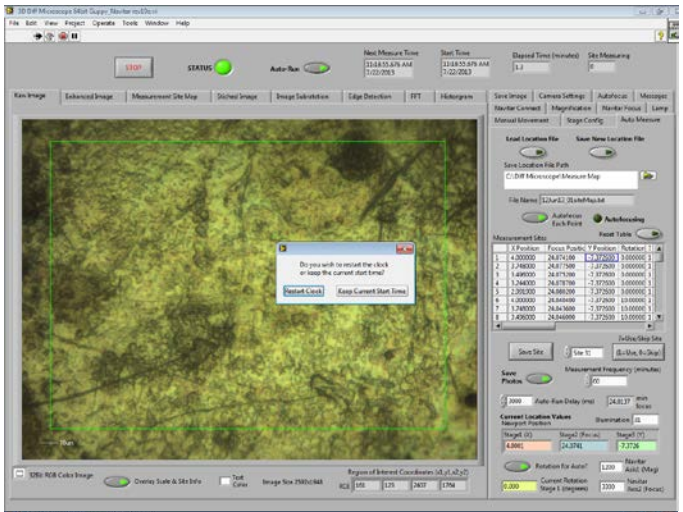
AUTO-RUN OPERATION

Although the system can acquire an image of any single location during manual operation, continuous acquisition of images from a large array of different site locations is the primary method for capturing sample data. Our goal is to continuously measure the differential change in the sample surface over a broad area and a prolonged time period. Automated measurement capability is mandatory for this goal. Figure 19 shows the user control panel view used during auto-run operation.

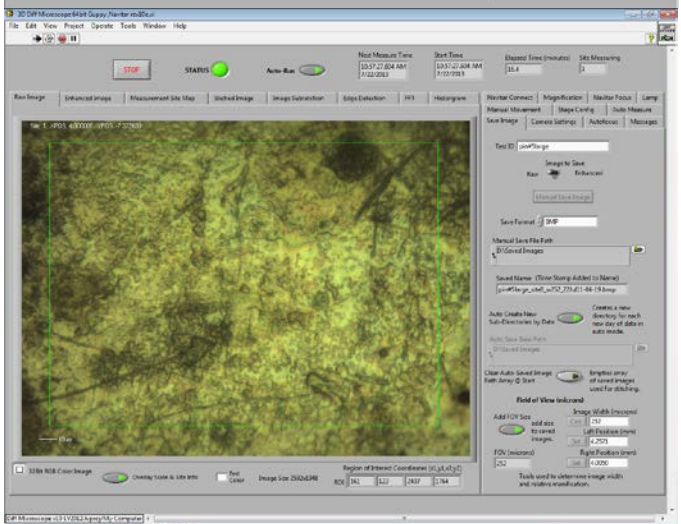
Auto-run is turned off by default when the image acquisition program begins. Prior to starting auto-run, the user should configure the 'Measurement Sites Table' of the 'Auto Measure' control tab panel shown in Figure 15c and Figure 19a. The sites should be arranged with slight overlap to both minimize area loss when a larger stitched image area is viewed and to view that maximum possible sample area during testing. The user can also load a previously saved table saved from tab delimited text file using the 'Load Location File' button. Individual sites may be added to this table by moving to the desired stage position coordinates, selecting the site number to assign, and pressing the 'Save Site' button. A site in the table may be skipped for measurement by toggle the '?=Use/Skip Site' to 0 (1=use site). Up to 100 sites may be saved in the array, but typically only 50 or 60 used because the measurement time for a single site is about one minute.

If the auto-run button is pressed while in manual mode (Figure 19a), a message box appears for setting the measurement start time by either restarting the clock to the present time or keeping the current displayed value from prior to the last auto-run stop/start. This feature is useful, if user wants to halt auto-run for a brief time and then resume without the elapsed time being altered by a new start time. In auto-run mode the current site being measured will be displayed as 'Site Measuring' and the elapsed time will be continuously updated. The entire array of sites will be measured at a frequency specified in minutes from a user input control with 60 minutes the default value. The user will typically want each site measurement autofocused to obtain the best quality image and the photo automatically saved, although these features can be disabled by button controls. During auto-run, images will be saved to a file name and directory specified in the 'Save Image' tab panel (Figure 19b). Images file names include the site number and a date-time-stamp. The image width in microns may also be added to the filename as an option. When testing has completed for the sample, auto-run may be ended by the user by pressing the (disabling) the auto-run button (Figure 19c), returning to manual mode. During operation the user, should periodically monitor the system for errors and warnings. Warnings are informational messages that may or may not indicate an issue that requires attention. These may include at failure to find an optimum focus position for a specific site or a GPIB communication time out message. Error messages should be given immediate attention and may indicate a camera problem, lamp error, or stage failure to move to the correct position. There is maximum system error to abort control that will automatically stop the program if this error limit is exceeded.

a



b



c

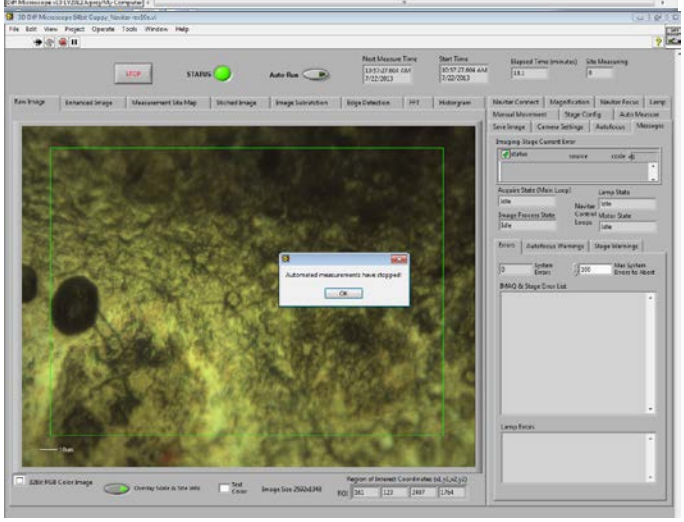


Figure 19: Auto-run operation panels showing (a) start of auto run with measurement sites auto-measure panel, (b) running with Autofocus panel visible, and (c) stop of auto-run with error/warning panel shown.

IMAGE STITCHING AND ANALYSIS

In addition to the image data view and control tab panels, there are a number of image processing and analysis tabs. These panels and image analysis capability can be used during auto run operation due to the semi-independent execution of loops that separate image acquisition from image analysis in the software. The operator can obtain preliminary image analysis information, such as defect size from a differential image, soon after the post image is acquired.

One of these tab panels, the image stitching tab (Figure 20) allows users to view a group of adjacent images arranged in rows in columns. These images are the most recently saved set of images acquired in auto-run mode. If no images have been acquired in auto-run mode, an error will be displayed when image stitching is attempted. This saved image array is typically emptied when the image acquisition program is started, but there is an option to use the array values from the previous run by disabling the 'Clear Auto Saved Image Path Array @ Start' button found on the "Save Image' control tab panel (see Figure 13a).

Images from the measurement site map are arranged into a 2D array of XY grid locations by site number. The first step for image stitching is to arrange the measurement site map positions array into XY grid positions (Figure 20a). When the 'View/Update Stitched Image' button is pressed (Figure 20b), the stitched image view is updated. The composite stitched image size may be varied between 2 to 4 images width and height (Figure 20b and Figure 20c). Individual images may be horizontally and vertically clipped / reduced (Figure 20d) by specified percentages to roughly align adjacent image edges. This alignment assumes the matrix of images is offset by similar X and Y distances / rotation offset across the matrix. During measurement site table setup, it is important that the X-Y or X-rotation positions are consistent for sites. Having a small offset in each direction will also minimize the overlap and loss of usable image area. The XY indices of the 2D image array corresponding to the top-left corner of the stitched image may be varied allowing the user to scroll (Figure 20e) across the stitched image area. If an invalid site is selected for display, an error message will appear in a popup dialog box (Figure 20f).

The stitched image produced in the image acquisition software provides a reference for a user to view an area larger than one camera image size and is not intended for detailed analysis. No stitched image save option is provided in this software. If high quality stitched images are needed, the user should use the LabView program for stitched image creation that includes illumination correction. Illumination correction is also absent for this simplified version of image stitching. Instead, post image acquisition software specific to image stitching should be used for image analysis. If too many features are provided, demands on system resources such as available RAM would too high and potentially compromise software reliability and responsiveness.

Image analysis capability includes subtraction of images of the same site taken at different time periods (Figure 21). The four sub tab panels include pre/post image selection, difference image, differential contrast, and defect size measurement. To the right of these panels are a number of controls for aligning the pre and post images.

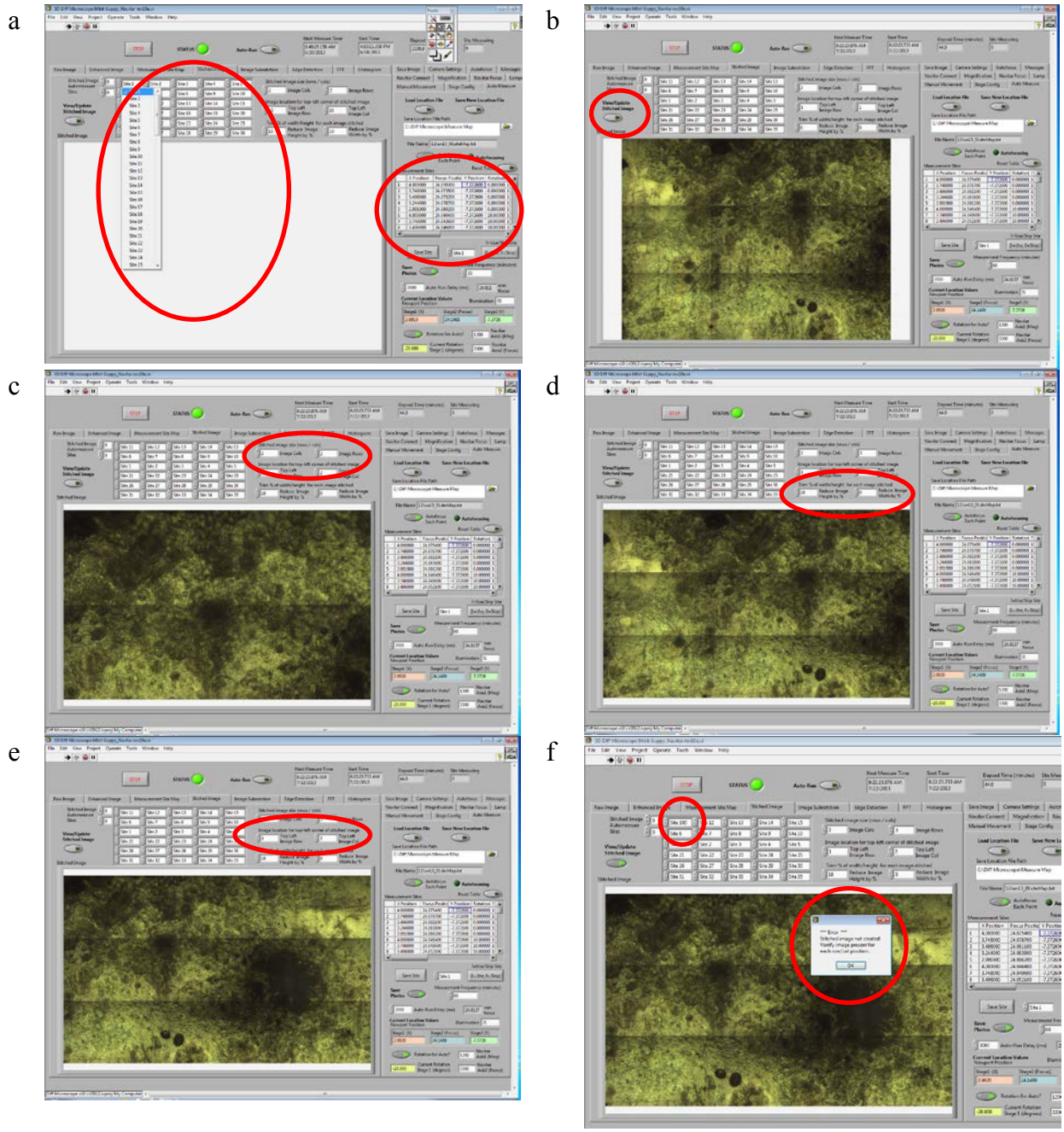
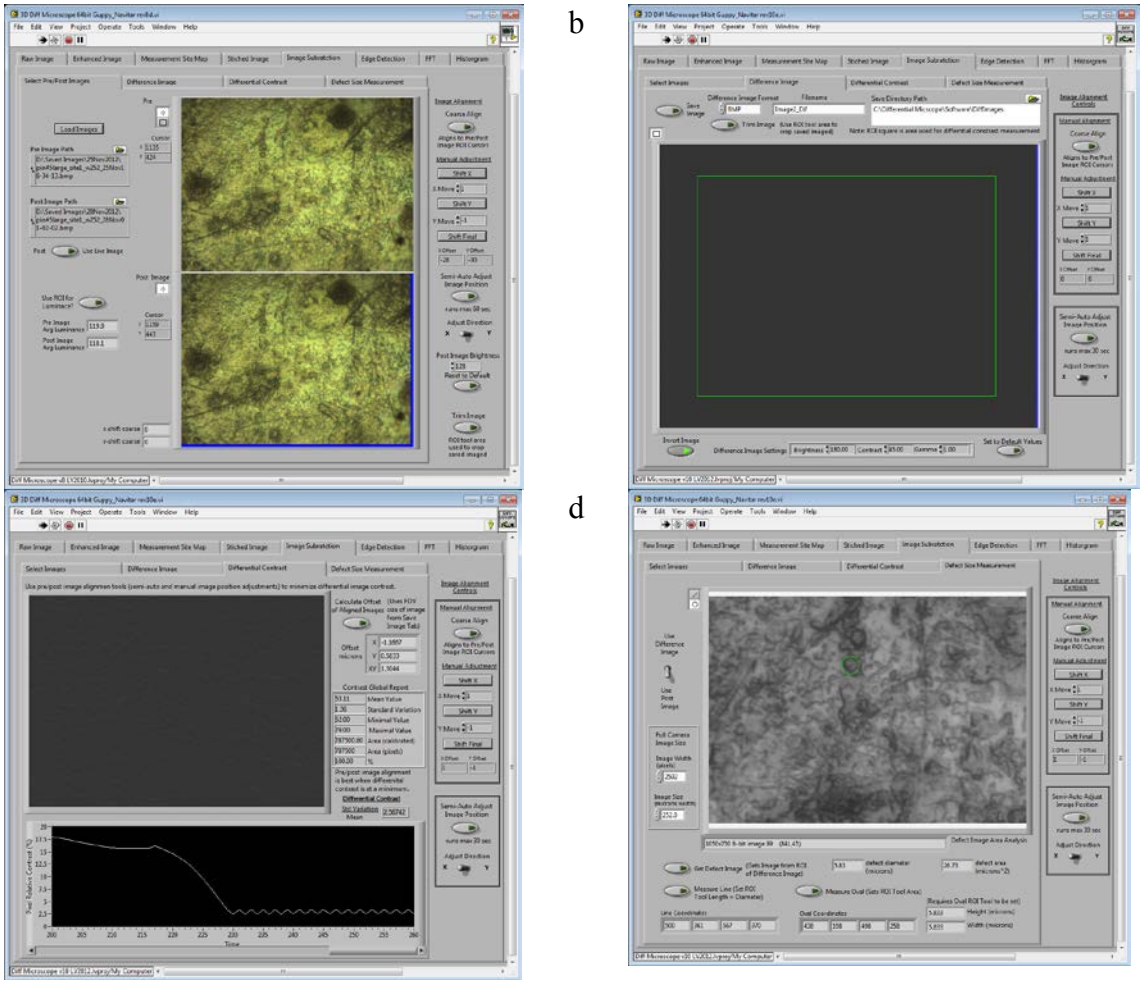


Figure 20: Image stitching tab panel showing (a) image array configuration, (b) stitched image display, (c) image size, (d) image trim , (e) image scroll, and (f) error message from attempting to display invalid image at site 100.

The pre and post images for subtraction are selected (Figure 22a) with the path browser controls or, for the post image, the current raw camera image may be selected by enabling the 'Post Use Live Image' button. The pre and post images may be coarse aligned by placing a crosshair cursor '+' on the same feature of both image and pressing the 'Coarse Align' button (Figure 22b). There are also indicators to measure and correct image luminance (Figure 22c) of entire images or a rectangular region of interest (ROI). The difference image panel shows the resultant subtracted image of pre image RGB values less post image values. Note that negative RGB

values are set to zero. The user can negate the subtraction values (displaying only the values that were negative) by enabling the 'Invert Image' button (Figure 22e). The difference image tab panel also includes a number of save options including image edge trim to ROI area, image save file type, save file path, and saved file name. The differential contrast panel is used for measuring image offset between the pre and post images. It includes a black & white view of the image area use for alignment. This area is determined as the selected ROI from the differential image panel (Figure 22d). The semi-auto adjust image controls are used to step the post image in either horizontal or vertical direction of X or Y movement increment in pixels to a minimal contrast alignment (Figure 22f). Included on this panel is a set of indicators for measuring the offset in microns between pre and post images. This offset distance is an important measurement to determine if significant image drift has occurred over time. Minimal image drift over an entire set of exposure images avoids having a large part of the subtracted image area be rendered useless. 5 microns drift per day is a maximum limit that should be allowed.

a b



c d

Figure 21: Image subtraction tab panels showing (a) pre/post image selection, (b) subtracted image, (c) differential contrast, and (d) feature size measurement.

The final sub panel for image subtraction is for defect size measurement. After successful pre/post image alignment, the operator may use either an oval or line tool to measure the size of

feature and estimate the feature equivalent diameter (Figure 23). The Guppy camera image size width in both pixels and microns must correct to accurately estimate the feature size. The operator should verify the values by confirming the horizontal number of pixels for the Guppy camera to be 2592 (if camera is rotated 90°, the vertical number of pixels should be used) and the image width in micron is determined by the method provided in Appendix E: Image Size Measurement.

To make a defect measurement, first select the image type to use by toggle switch for either difference or post image. Press the 'Get Defect Image' button to image the image display. Select either oval or line tool and place it on the feature to measure measuring either an outline of the defect with the oval tool or the diameter with line tool. When the measure line or measure oval button is pressed the estimated feature diameter and area are displayed on the indicators in the bottom right section of this panel. For the oval measurement, the area is the measured area of the oval using the oval coordinates listed at the panel bottom. An average diameter is calculated assuming a circle of similar area. For the line measurement, the estimated diameter is the length of the drawn line, and the area is estimated as the area of a circle with the same diameter.

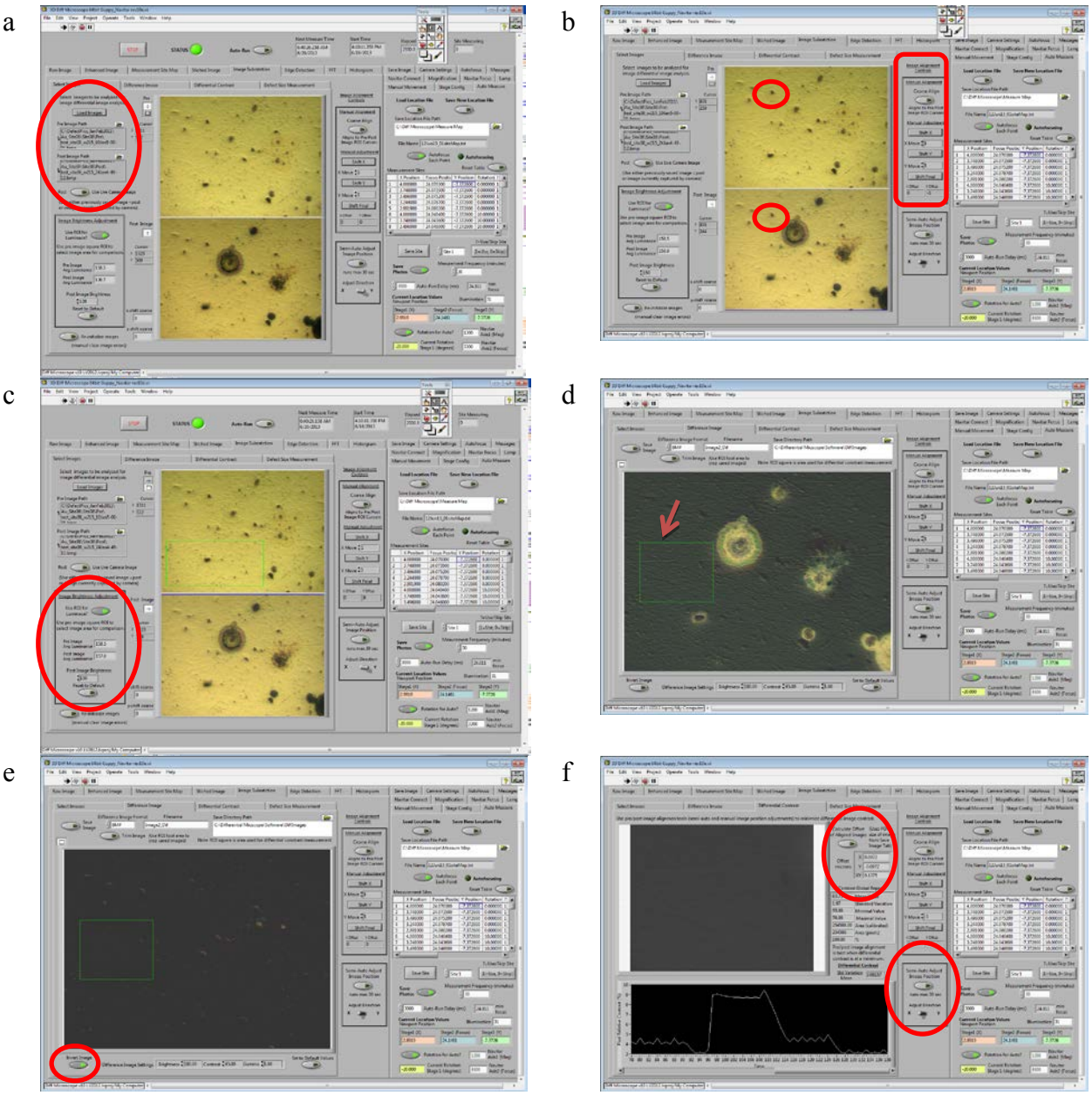
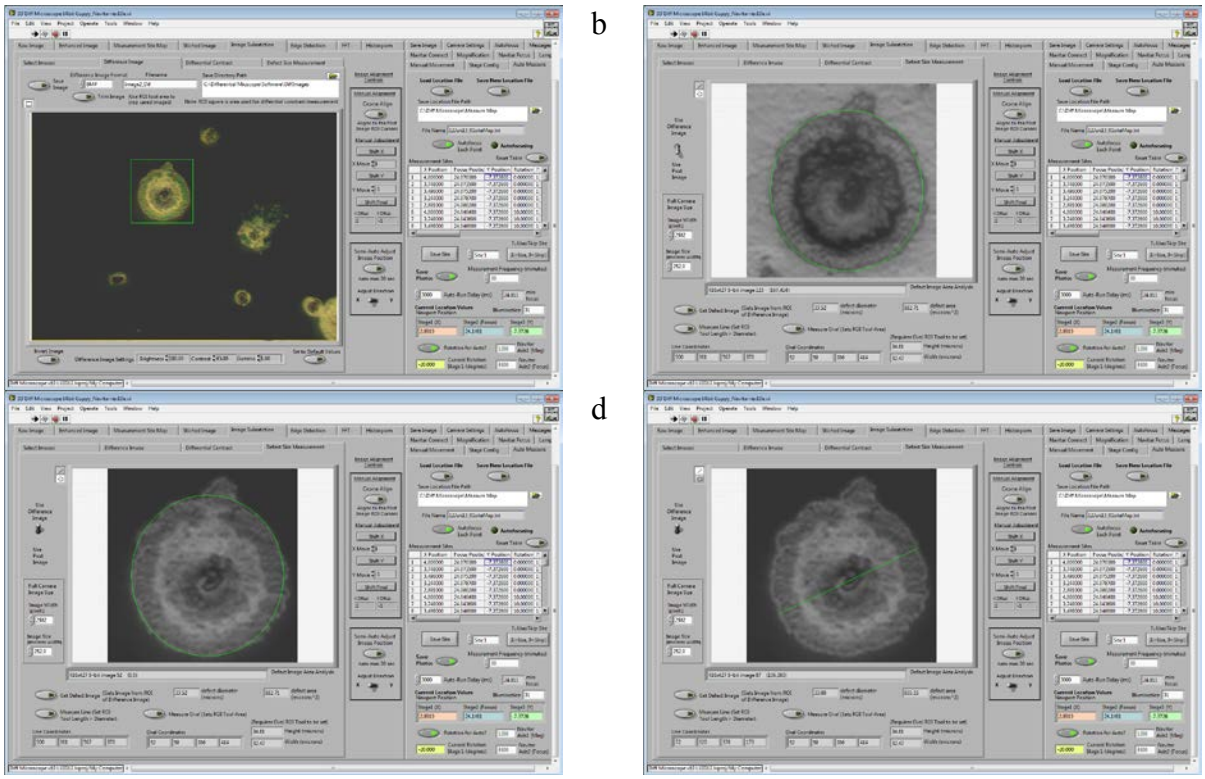


Figure 22: Image alignment showing (a) image load selection, (b) coarse alignment, (c) luminance correction, (d) subtracted image view with alignment ROI selection, (e) inverted image, and (f) image alignment by minimizing differential contrast and offset distance.

a b



c d

Figure 23: Defect size meausrement showing (a) defect location selection, (b) post image view, and (c) different image view. Defect measurement by (c) Oval size versus (d) line size is shown.

SYSTEM OPERATION

Operation of the differential microscope is covered in the operating procedure: *OP Differential Imaging Microscope.* The instructions that follow are those operation procedure instructions with additional references to sections of this software reference document.

1) Power Up (Initial conditions)

- **a.** Verify the sample chamber is isolated from the gas exposure system. If not isolated, open the bypass valve and close the return/supply valves (see Figure 7b).
- **b.** Turn on the power switch to both Newport ESP301 stage controllers.
- **c.** If not already at home positions, use the ESP301 menu panel to return all Newport stages to their home positions (see Appendix H). First, move the focus stage to home to avoid collision with the microscope objective, and follow by moving all other stages to their home positions.
- **d.** Turn on the Navitar motor control module power switch.
- **e.** Turn on the Navitar lamp control module power switch.
- **f.** Turn on the Digi-Sense temperature controller rear power switch. Verify the temperature controller is in 'stop' mode.

2) Computer and Software Startup

- **a.** Turn on the image acquisition system computer and monitors.
- **b.** Start the National Instrument's Measurement and Automation Explorer (NI-MAX) program (see Appendix A). With NI-MAX confirm computer communication to the camera via IMAQ and ESP301 controller(s) via GPIB. Close NI-MAX.
- **c.** Start the Navitar Serial Motor Control Program 'Navser.exe' for Windows (see Appendix B). Set COM1 as the communication port in software. Home the magnification and focus motors. Cycle both motors to their maximum movement range and re-home. Confirm the maximum range is 2123 +10 steps for magnification and 6775 + 25 steps for focus. Verify the motors move to a desired preset position (3300 steps for focus and 1200 steps for magnification). Close the motor control software program when finished.
- **d.** Start the Navitar Lamp Control LabView program 'LampSer64.vi'. Set COM2 as the communication port in software (see Appendix B). Verify the lamp illumination changes with varying set point. Close the lamp control software when finished.

3) Operation Setup

- **a.** If system errors are reported during operation, contact the equipment owner and determine if corrective action is needed.

b. Starting a New Sample or Reconfiguration of the Sample Chamber.

c. Move the stages to positions where the sample chamber has sufficient clearance from the Navitar microscope objective using the ESP front panel controls or joystick controller.

d. Turn off power to the ESP301 controllers.

e. Turn off power to the Digi-Sense temperature controller, and disconnect the heat tape from the controller.

f. Verify the bypass valve is open and the isolation valves to the GES are closed.

g. Disconnect the GES inlet and outlet tubing from the sample chamber.

h. Remove the sample chamber from the rest of the XYZ stage assembly taking care not to damage any cables.

i. Remove the screws holding the sample chamber together and remove the quartz viewport. G ently clean the quartz viewport with a clean wipe and, if needed, Alconox.

j. If a sample is present, remove the sample from the chamber. Clean the chamber and sample holder with clean wipes and, if needed, Alconox.

k. If switching between flat coupons and curved pin samples, reconfigure the sample chamber and sample holder for the new sample type.

l. Place the new sample in the holder. If a curved pin, place the sample in the rotation shaft grove (Figure 6e). If a flat coupon, mount the sample to the sample block with the clamps (Figure 6d). Verify the sample is firmly held in position.

m. Reassemble the sample chamber and remount the quartz viewport (Figures 6a-6c). If a pin sample, verify that the rotation shaft is centered in the chamber isolation seal and firmly attached to the rotation stage via set screws.

n. Mount the sample chamber back on the XYZ stage assembly.

o. Visually inspect the sample chamber thermocouple, and replace if damaged. Verify it is attached with Kapton tape to the side of the chamber.

p. Turn on t he rear power switch to the Digi-Sense temperature controller. Keep the temperature in stop mode (unheated state). Verify the temperature displayed is room temperature (20-25°C). Turn off the rear power switch to the temperature controller.

q. Wrap the heat tape for chamber temperature control around the outer side walls of the sample chamber, taking care not damage or dislodge the thermocouple (Figure 6c). Plug the heat tape power connector into the rear of the temperature controller.

r. Turn on the rear power switch to the Digi-Sense temperature controller, but keep the temperature in standby (unheated state). Verify the room temperature is displayed.

s. Turn the power back on to the ESP301 controllers and home all stages.

t. Using the ESP301 XYZ stage front panel controls or joystick controller, move the stages to positions where the sample chamber front window and sample are positioned in front of the Navitar microscope objective.

u. Verify the Digi-Sense temperature set point is 35°C, and adjust if needed. Heat the sample chamber by pressing the 'Start' button on the temperature controller. Check the chamber is heating to 35°C, and allow 2 hours for the temperature to stabilize.

v. Connect the gas exposure system inlet and outlet tubes to the sample chamber (Figure 6a). Do not open the inlet/outlet valves or close the bypass values (Figure 7b).

4) Manual Operation and Measurement Site Configuration

a. If not already powered on, turn on power to the Navitar motor and lamp controllers. If the computer or Navitar motor controllers have been turned off at any time during prior work, perform the steps from 'Computer and Software Startup'.

b. Open LabView and start the program for differential imaging microscope image acquisition as per the 'Getting Started' section. Keep the program in manual mode which is the default at program start.

c. Verify the Navitar microscope motor and lamp controllers are detected by the LabView program ('on-line' indicated on Figure 14a and Figure 14d). Home the focus and magnification motors (step 0 positions on Figure 14b and Figure 14c). Adjust focus position to 3300 steps and leave magnification at 0 steps (lowest magnification).

d. Adjust the Navitar lamp control intensity until a bright region is visible in the raw image area. If no bright region is seen, visually inspect the sample surface to see if the illumination spot is hitting the sample area. Adjust the X (horizontal) and Y (vertical) stage positions from the manual stage movement tab (Figure 15b), until the illumination spot is visible on the sample surface and on the raw image.

e. Adjust the vertical (Y stage) position from the manual stage position controls (Figure 15b) until the bright region is centered in the raw image area.

f. Using the manual stage position controls (Figure 15b) move the focus distance (Z stage) to position the sample surface at the working distance away from the microscope objective. Measure this working distance with a ruler (working distance is 20mm for the 20x or 33mm for the 10x Mitutoyo objectives).

g. Adjust focus (Z stage) position until the image until the image is no longer blurred. The vignette at the image edges will still be present, because the image will be at minimum magnification.

h. Step the magnification motor control to the desired magnification (Figure 14b). The typical magnification motor setting is 1200 steps which correspond to an image width of approximately 250 microns.

i. Adjust the vertical position until the bright region is centered in the 'Raw Image' area. Manually adjust the focus position until the image until the image is in focus.

j. Verify the settings for autofocus (Figure 17): (1) set the desired focus area region of interest (ROI) in the raw image area, (2) select 'Use ROI' on/off as desired from the Histogram tab for coarse focus, and (3) set FFT for fine focus method on the Autofocus tab.

k. Confirm the desired fast Fourier transform settings are selected for fine focus (Figure 17). Run a single point FFT acquisition.

l. Press 'Run' for Auto-focus manual operation (Figure 13c). Verify combined coarse and fine image focus is achieved in < 40 focus attempts.

m. Verify the image edges (top/bottom/left/right) are all in focus or, if a curved pin sample, the curved edges (top/bottom) achieve focus at the same z-position. For curved edges, the Y stage position should be manually adjusted by software to align center the camera image to the middle of the pin sample surface. If non-curved edges are not in focus, sample or stage mechanical adjustment may be needed to achieve perpendicular sample alignment to the microscope objective. If mechanical adjustments are required, the user will need to complete repeat the steps from 'Starting a New Sample' and/or all previous steps from this section 'Manual Operation and Measurement Site Configuration'.

n. If sample alignment, focus, and position are satisfactory, save the current stage positions to the measurement site table on the configure auto-measurement tab (Figure 15c). If old sites are listed from a previous experiment, delete these sites by pressing 'Reset Table' prior to saving the new site. Set this site as the sample center by pressing the 'Sample Origin' button on the Measurement Sites tab (see Appendix G).

o. Camera color accuracy may be checked using a Munsell mini color checker chart. If adjustment is needed, contact the equipment owner prior to attempting any adjustment and follow the instructions in the Differential Imaging Microscope System Acquisition Software Reference Appendix C to verify correct color balance.

p. To estimate microscope magnification, measure the image width in microns as per Appendix E. Locate an artifact on the sample surface, move it to the left edge of the

image, and record the stage position by pressing the 'Set' button for left position. Move the artifact to the right edge and record this position with the right 'Set' button. Press the 'Calc' button to calculate the image width in microns.

q. Move the sample XYZ and rotation settings to the next site for measurement. Achieve sharp focus on this site, and save it to the measurement sites table on the configure auto-measure tab using a new site # (Figure 15c).

r. Repeat the previous step to produce an array of site locations saved in the measurement sites table. Up to 100 site locations may be saved. When creating this table, try to arrange image locations so that the images edges overlap to facilitate mapping over a large sample surface area.

s. View the site locations from the measurement sites tab display (Figure 12c and Appendix G). Select the sample type (curved pin or flat coupon). Press the 'Load Sites from 'Auto Measure' button to display the array of sites on the grid map.

t. Set the sample perimeter area on the measurement site map (Appendix G). For flat coupons the sample width / length are entered; the XY stages are moved to the top-left, top-right, bottom-right, bottom-left corners of the sample; and those XY stage positions entered on the measurement site map page. For curved pin samples, the sample maximum rotation angle, sample length, and min/max X stage positions entered. Press the 'Draw (Curved/Flat) Sample Region' button to display the sample perimeter in red.

u. If the sample measurement sites are satisfactory, save those site locations to a tab delimited text file by pressing the 'Save New Location File' button (Figure 15c). The saved file path is set by the file path control. The file name is the saved Date-Time stamp appended with 'siteMap.txt'. Your sample is now ready for automated measurement.

5) Automated Measurement

a. Prior to exposing a test sample to corrosive gasses, the sample surface must be baselined by collecting pre gas exposure images. Do not close the bypass value or open the GES inlet/outlet values (Figure 7b) to the sample chamber prior to completion of the steps from Section 4 - Manual Operation and Measurement Site Configuration.

b. Verify the measurement site table values are correct for the sample to be measured (Figure 15c). If the table values are incorrect, previously saved site table values from the text file saved in step 7.4.3.20 may be used. Load these saved values in to the site map table by pressing the 'Load Location File' button and navigate with the browse window to the file. If the correct site locations cannot be found, repeat the measurement site setup steps from Section 4.

c. View the site locations from the measurement site map display (Figure 12c) Select the sample type (curved pin or flat coupon). Press the 'Load Sites from 'Auto Measure' button to display the array of sites on the grid map (Appendix G).

d. Move to a site by selecting the site number and pressing the 'Move to Auto-measure Site #' button (Appendix G). Verify the XYZ and rotation stage positions for that site are identical for the measurement site table, current location values display, and on the ESP301 front panel displays. Verify that sample surface is in focus and, for curved pins, centered in the image area. Repeat this step for at least three different sites at different X and Y (flat coupon) or rotation (curved pin) positions.

e. From the 'Save Image' tab (Figure 13a), update the Test ID to name for the experiment being run. Verify the auto save file path is set to "D:\Saved Images\".

f. Confirm that > 400GB of hard disk space is available for saving images on the D drive. If less free space is present, save old image data for deletion to an external hard disk and delete those old files present from the D drive. Disconnect the network Ethernet cable from the back of the computer to prevent the computer program from being interrupted during auto operation.

g. Verify the sample is moving to the correct locations and autofocus is consistently achieved.

h. Verify image data is being saved to the directory "D:\Saved Images\" + current date.

i. Allow the system to run overnight (> 12 hours). Verify focused images were successfully saved for all sites over that time period.

j. Analyze 3 or more selected images for positional drift (Appendix F). This measurement is made using the procedure in appendix F. Drift of 5 microns or less in the X and Y directions should be demonstrated for all images examined. If a larger drift is observed, this is likely sample movement over time rather than random variation in stage position. The sample may have to reposition/re-clamped in the sample chamber.

k. If sharp focus and stable position of the sample surface are achieved, the sample is ready for exposure to corrosive gasses. Press the 'Auto-run' button to temporally halt measurements (the button should no longer be lit green and dialog box will prompt that the auto measurements have stopped). Open the GES inlet and outlet value to sample chamber, and close the bypass value Figure 7b). Press the 'Auto-run' button again and select 'Reset Start Time' (see Auto-Run operation). Confirm the 'Auto-run' button is lit green, indicating automatic operation.

l. Verify the system is moving to the correct locations, achieving sharp image auto focus, and saving images to the correct directory. Analyze 2-3 images for position drift as was done previously (Appendix F).

m. Repeat image drift analysis on a periodic basis (every 12 to 72 hours) for the duration for the corrosion experiment (typically 14 days).

n. After completion of the experiment, return the LabView program to manual mode by pressing the 'Auto-run' button, and verify that button is no longer lit green.

o. Press the LabView program 'Stop' button. Open the gas bypass value, and close the GES in/out isolation valves. Turn off the Digi-Sense temperature controller.

p. Reconnect the computer to the network by plugging in the Ethernet cable. Transfer image data to the image analysis computer.

6) Shutdown

a. If the LabView program is still running, first stop auto-run (if not in manual mode) and then press the program 'Stop' button. A fter the program ends, close the differential imaging microscope program/project and exit LabView.

b. Turn off power to the temperature controller.

c. Isolate the sample chamber from the gas exposure system by opening the bypass valve and closing the return/supply valves.

d. If not already at home positions, use the ESP301 menu panel to return all Newport stages to their home positions (Appendix H). First, move the focus stage (z-direction) to home to avoid collision with the microscope objective, and follow by moving all other stages to their home positions.

e. Turn off the power switch to both of the Newport ESP301 stage controllers.

f. Turn off the Navitar motor control module power switch.

g. Turn off the Navitar lamp control module power switch.

h. Perform steps 7.5.8-10 for extended shutdowns (such as Winter Break)

i. Turn the computer and monitors off.

j. Turn off the system power strip.

k. Unplug the electrical cords for the UPS and power strip.

APPENDIX A - NI-MAX (NATIONAL INSTRUMENTS MEASUREMENT AND AUTOMATION EXPLORER)

Measurement and Automation Explorer (NI-MAX) is a s oftware application available for National Instruments for configuring and verifying software communication to instrumentation. Ni-MAX may be started either from the Windows program 'Start' button with 'National Instruments / Measurement & Automation Explorer' or from a desktop shortcut. There different types of instrument communication are used for the image acquisition system: GPIB, RS232 serial, and Fire-wire/IMAQ.

The two ESP301 stage controllers use GPIB (General Purpose Instrument Bus) parallel communication with the IEEE1488.2 standard. GPIB address 1 is assigned to the ESP301 XYZ linear stage controller, and GPIB address 2 assigned to the second ESP301 controller for rotation stage(s). A National Instrument's USB-GPIB converter box is provides the connection between each ESP301 controller and the computer. GPIB address assignment can be confirmed by selecting "Devices and Instruments / GPIB-USB-HS" and pressing "Scan for Instruments" at the top/middle of the program window (shown in Figure A1).

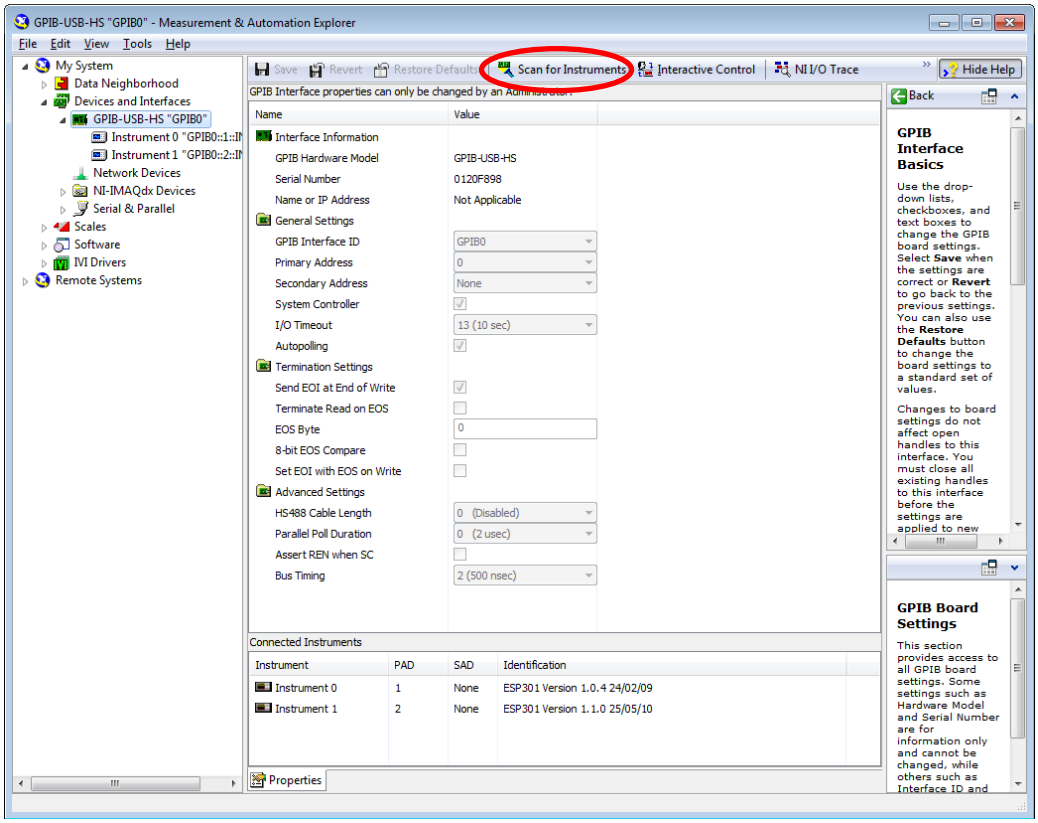


Figure A1. GPIB instruments configuration shown in MAX.

The Navitar stepper motor controller and lamp illumination controller use RS232 serial communication. Stepper motor controller for microscope magnification and focus is assigned to serial COM port 1 (motherboard com port), and illumination control is assigned to serial COM

port 2 (USB to RS232 device). The Navitar controllers may also be controlled by USB, but RS-232 software drivers (as a DLL files) are not available in 64-bit. The older 32-bit USB driver files are not compatible with 64-bit LabView. Configuration of the RS232 serial ports may be viewed in MAX (Figure A2).

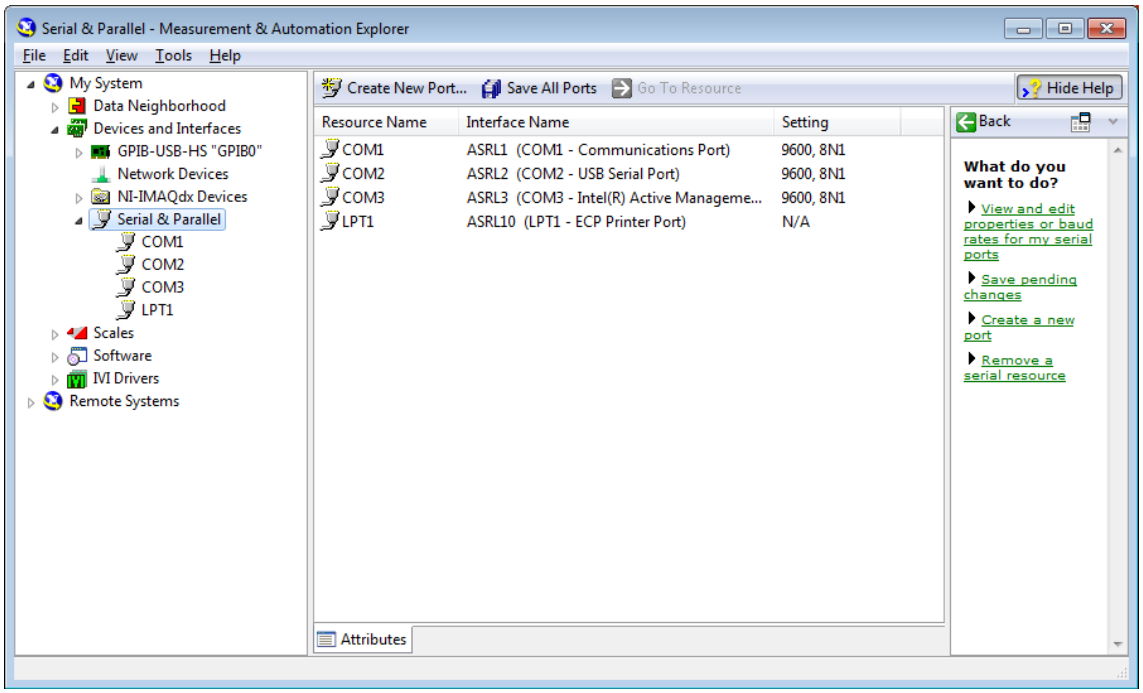


Figure A2. RS232 serial port configuration shown in MAX

Camera control is via a firewire.b interface cable between the Guppy camera and a PC controller board. The IMAQ (Image Acquisition) software driver for the camera is provided by the NI Vision Acquisition Software. The IMAQ setting for the camera are displayed in the NI-MAX and should be verified by the image acquisition system user prior to running a new experiment. The AVT Guppy F503 camera should be configured as cam2 (previously a PixeLINK camera was configured as cam0 and cam1 depending on manufacturer firmware version). Figure A3 shows the camera configuration default settings. The setting can be altered, but any changes should first be verified with the equipment owner. Modifying these settings may result in serious problems such as incorrect image color or poor image contrast. The Guppy camera should be configured as Format 7.

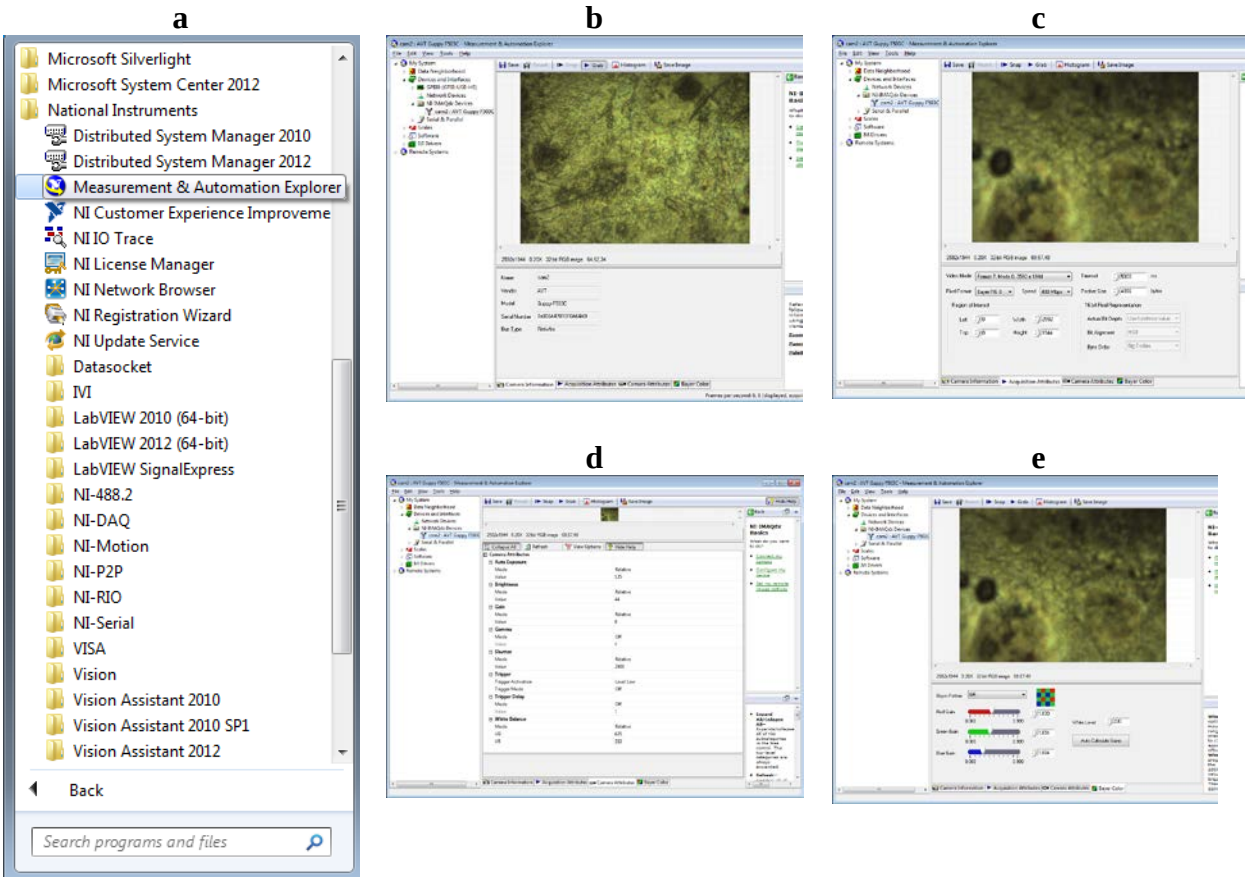


Figure A3. Measurement and Automation Explorer (MAX): Navigation to program using (a) Start / Programs Tree menu. NI-IMAQ camera configuration in MAX showing (b) camera information, (c) acquisition attributes, (d) camera attributes, and (e) Bayer color correction.

APPENDIX B - NAVITAR MICROSCOPE MANUAL CONTROL SOFTWARE

The Navitar 12x Ultrazoom microscope uses two different sets of control hardware: one for focus/magnification control and the other for illumination control. Although this hardware has been previously described in the *System Hardware Overview* section, this appendix provides additional information for controlling this hardware. Both RS232 serial and USB communication control is available for Windows computer control, but only RS232 is supported for 64-bit programs such as LabView 64-bit. If USB connections are preferred for manual operation, the user may connect the controllers to a USB port and use the 32-bit USB version of the controller software. This section describes the software provided by Navitar for operating either set of hardware outside of the Differential Image Acquisition Software written by Sandia.

Control of microscope magnification and focus is through two 5-phase stepper motors connected to a power supply controller that can be connected to a Windows computer. 32-bit Windows software is provided by Navitar and runs from the Program Files (x86) directory. Both Serial (RS232) and USB versions of this program are available from 'Start->Programs'. Figure B1 shows the navigation path and user interface for these programs. It is recommended that the Serial version of the program be used, because RS232 is the only communication method supported by LabView 64-bit. When starting this program, a panel appears that shows the step positions of both motors. Detailed instructions for operated this software is available from the *Navitar Motorized Zoom Systems Instruction Manual for Windows*. When starting the stepper motor controller, the user should verify the movement range accuracy of the both the focus and magnification stepper motors. Cycle both motors to their maximum movement range and re-home. Confirm the maximum range is 2123 $\pm$10 steps for magnification and 6775 $\pm$ 25 steps for focus. Verify the motors move to a desired preset position (3300 steps for focus and 1200 steps for magnification. Additionally, at 1200 steps magnification the, image width with 5MP Guppy Camera should be 250 +/- 25 microns.

Manual control of LED illumination source is either through the rotary dial at the top of the lamp controller or using LabView 64-bit with the program *LampSer64bit.vi* (Figure B2). Please note that the image acquisition LabView program should not be run simultaneously with this manual lamp control program or a hardware conflict error will result. COM2 should be the serial port configured for this controller. Control of lamp brightness from 0 to 100% lamp output is via a slider bar. An indicator displays the current output brightness.

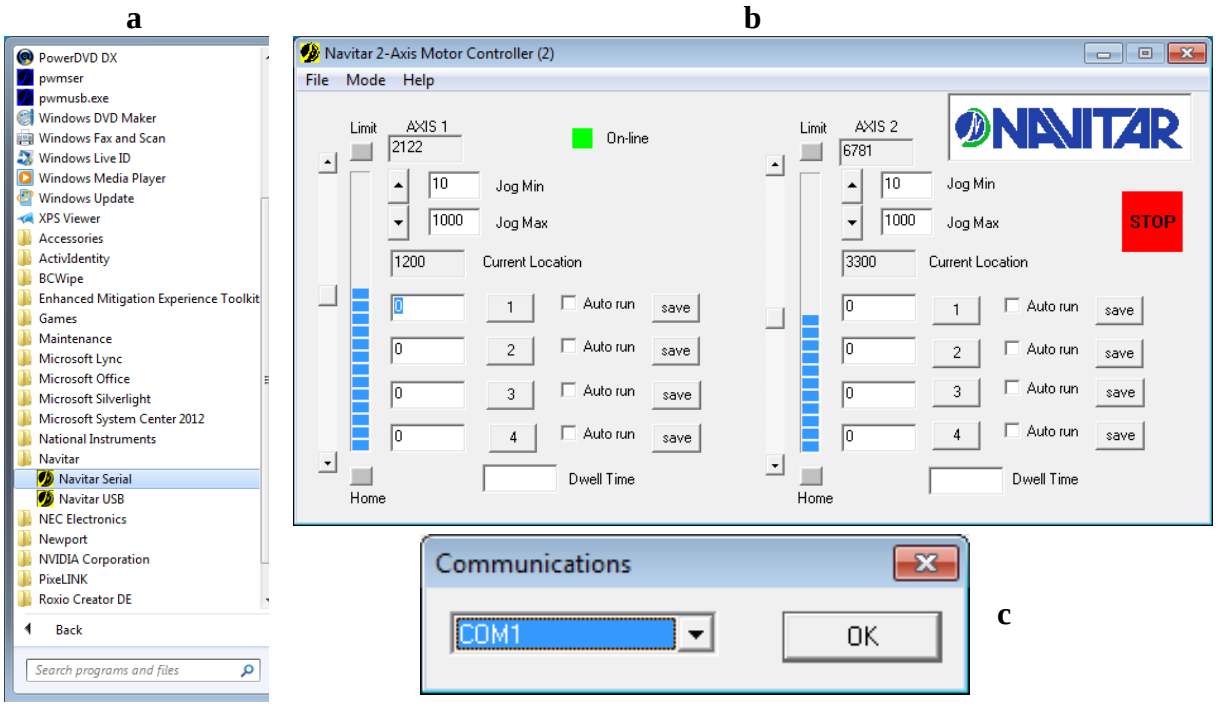


Figure C1. Navitar motor control (a) serial control location from 'Start->Programs Menu', (b) program user interface, (c) RS232 serial communication port COM1.

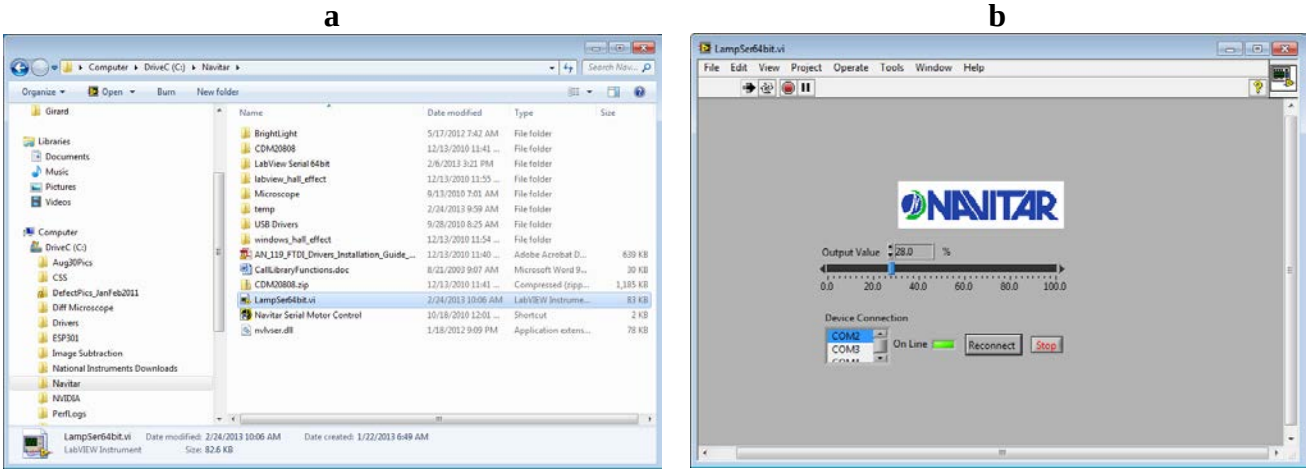


Figure C2. Navitar lamp illumination manual control software. Program file path (a) and LabView program front panel with serial port configured for COM Port 2(b).

APPENDIX C – MICROSCOPE ZOOM RANGE CHECK

All Navitar zoom systems are designed to stay in focus when adjusted from one magnification to another. The procedure below describes how to adjust the components.

1. Position fine focus mechanism at the middle point of travel. (If the lens has fine focus.)
2. Adjust the magnification to its highest setting.
3. Adjust focus by either moving entire optical system in its z-axis or using the fine focus provided on the lens. Adjust until you have a sharp image.
4. Adjust the magnification to its lowest setting. At this point do not re-adjust z-axis height or the fine focus on the lens.
5. Adjust the back focus of the lens. This is done by adjusting the glass in the adapter tube. Most adapter tubes with glass have a locking screw and an adjustment screw. Release the locking screw and then use the adjustment screw to bring the image into focus. If using adapters without glass they can be adjusted using the telescoping feature or the CCD in the camera can be adjusted.
6. Lock the back focus adjustment.

At this point the lens should produce sharp images from low to high magnification.

APPENDIX D - COLOR CALIBRATION

A color calibration check may be performed at this time by attaching a MacBeth/Munsell mini color checker chart (Figure D1) on the outside of sample chamber or stages. Correcting color gain settings should be done with caution and, if problems occur during setup, the system owner should be contact for assistance. This process has not been fully developed and will require experimentation by the user to achieve a satisfactory result.

a

b

c

No.	Number		sRGB R	G	B	CIE L*a*b* L*	a*	b*	Munsell Notation Hue Value	Chroma
1.	dark skin		115	82	68	37.986	13.555	14.059	3 YR	3.7 / 3.2
2.	light skin		194	150	130	65.711	18.13	17.81	2.2 YR	6.47 / 4.1
3.	blue sky		98	122	157	49.927	-4.88	-21.925	4.3 PB	4.95 / 5.5
4.	foliage		87	108	67	43.139	-13.095	21.905	6.7 GY	4.2 / 4.1
5.	blue flower		133	128	177	55.112	8.844	-25.399	9.7 PB	5.47 / 6.7
6.	bluish green		103	189	170	70.719	-33.397	-0.199	2.5 BG	7 / 6
7.	orange		214	126	44	62.661	36.067	57.096	5 YR	6 / 11
8.	purplish blue		80	91	166	40.02	10.41	-45.964	7.5 PB	4 / 10.7
9.	moderate red		193	90	99	51.124	48.239	16.248	2.5 R	5 / 10
10.	purple		94	60	108	30.325	22.976	-21.587	5 P	3 / 7
11.	yellow green		157	188	64	72.532	-23.709	57.255	5 GY	7.1 / 9.1
12.	orange yellow		224	163	46	71.941	19.363	67.857	10 YR	7 / 10.5
13.	blue		56	61	150	28.778	14.179	-50.297	7.5 PB	2.9 / 12.7
14.	green		70	148	73	55.261	-38.342	31.37	0.25 G	5.4 / 8.65
15.	red		175	54	60	42.101	53.378	28.19	5 R	4 / 12
16.	yellow		231	199	31	81.733	4.039	79.819	5 Y	8 / 11.1
17.	magenta		187	86	149	51.935	49.986	-14.574	2.5 RP	5 / 12
18.	cyan		8	133	161	51.038	-28.631	-28.638	5 B	5 / 8
19.	white (.05*)		243	243	242	96.539	-0.425	1.186	N	9.5 /
20.	neutral 8 (.23*)		200	200	200	81.257	-0.638	-0.335	N	8 /
21.	neutral 6.5 (.44*)		160	160	160	66.766	-0.734	-0.504	N	6.5 /
22.	neutral 5 (.70*)		122	122	121	50.867	-0.153	-0.27	N	5 /
23.	neutral 3.5 (.1.05*)		85	85	85	35.656	-0.421	-1.231	N	3.5 /
24.	black (1.50*)		52	52	52	20.461	-0.079	-0.973	N	2 /

Cie L*a*b* values use Illuminant D50 2 degree observer sRGB values for Illuminate D65.

Figure D1. Munsel (MacBeth) color checker chart (a) with numeric codes for each color (b) and RGB values for each color code.

Taking care to avoid collision with the microscope stage, move the stage XYZ positions to the center of color chart site #12, which is orange/yellow color (see Figure D2). Record these XYZ positions for future reference. From the camera setting tab, set target color to red with a value of 224. The measured green and blue intensities should be set to values that match earlier measurements. Although the Munsel charts shows that green should be 216 and blue intensity 46, these value assume a broad white light (sun) spectrum rather the LED light source with a polarizing filter(s). Typical measurement of color accuracy standard uses camera configuration settings, good LED illumination, and a defocused image of color chart site #12. Using a target red value of 224, green is measured at ~ 165 and blue is measured at ~ 46. The white Balance:UB(lue) and White Balance:VR(ed) setting may be adjusted if the image color is

incorrect. The values will be programmed into the camera, adjusting the raw image color balance.

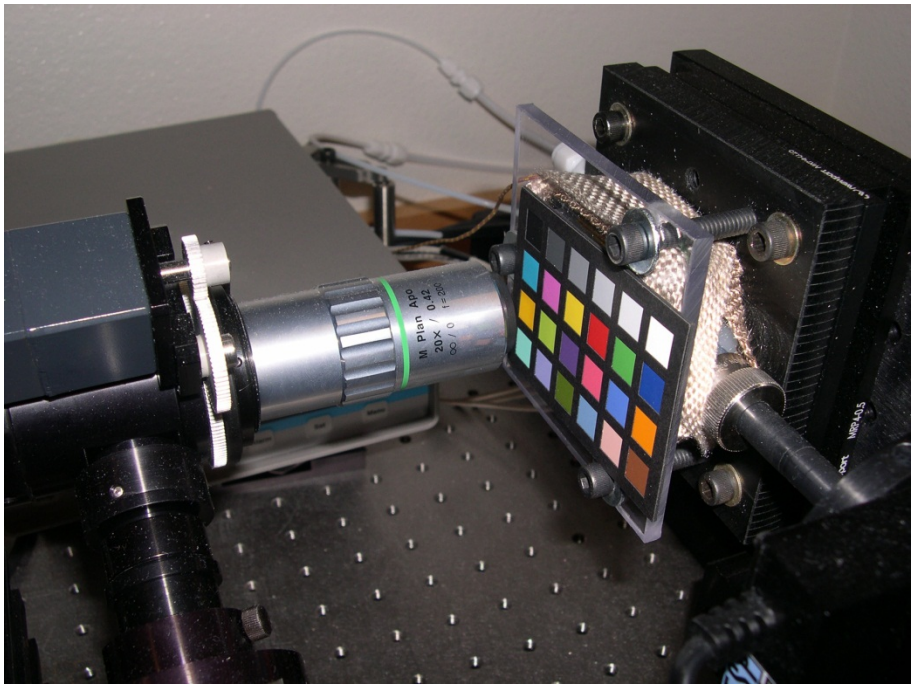


Figure D2. Munsel mini color checker chart positioned in front of object for color calibration measurements.

An alternative check for color accuracy is to compare color balance values for a test sample to previous color balance values for either the same or another sample. Figure D4 shows color balance values for a sample in focus. These measurements could also be made for a defocused sample surface. These color balance measurement and correction techniques are still under development. Additional approaches may be possible and no one method is deemed the sole method for use.

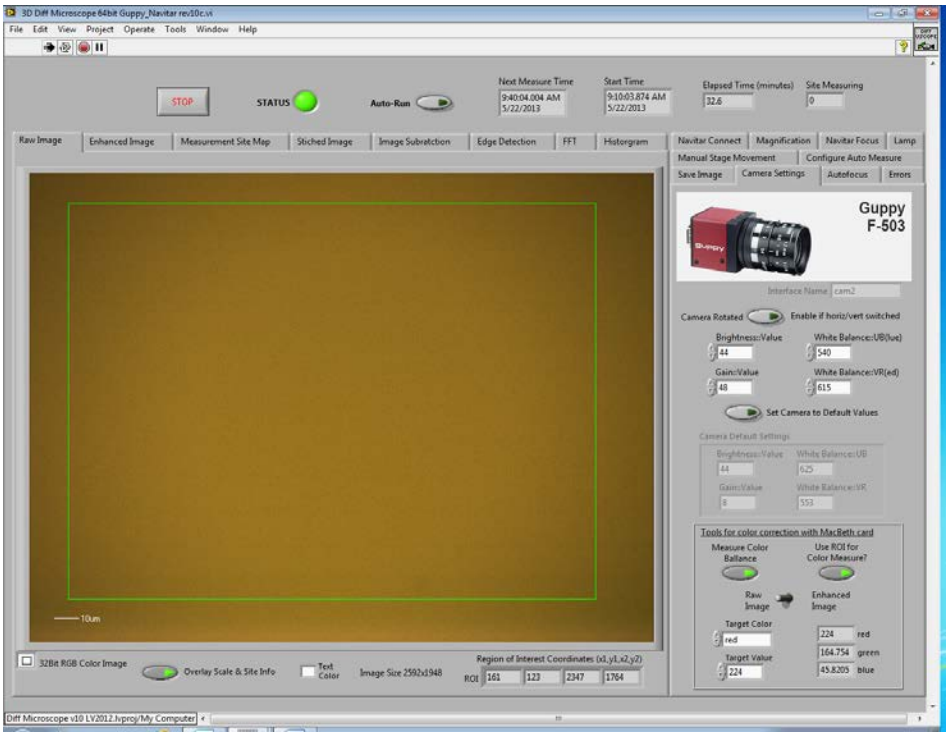


Figure D3. Defocused image of color chart site #12 showing RGB values measured at default camera settings.

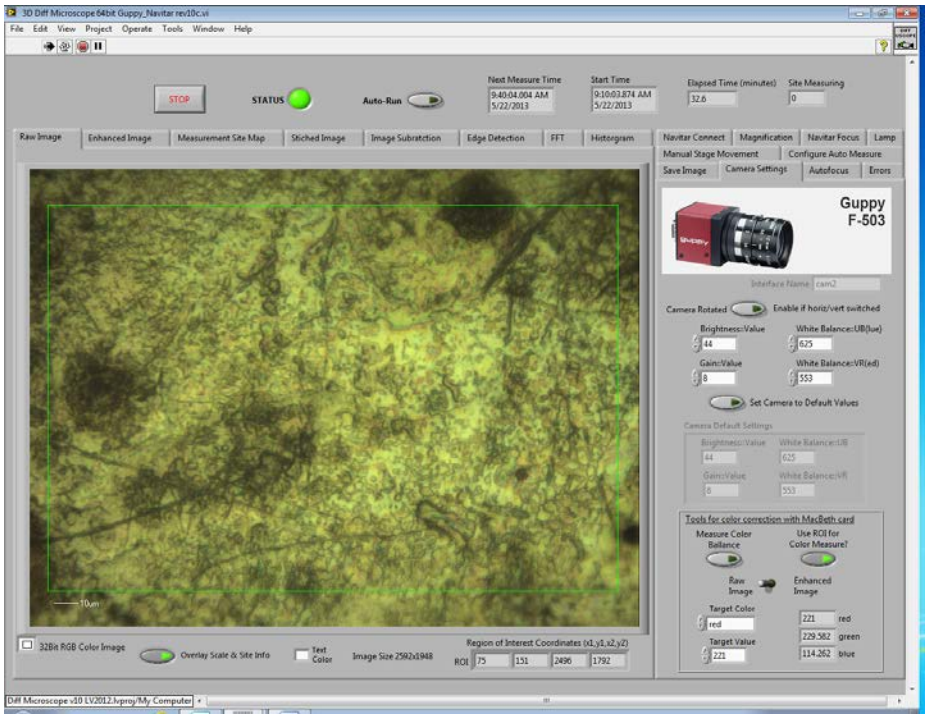
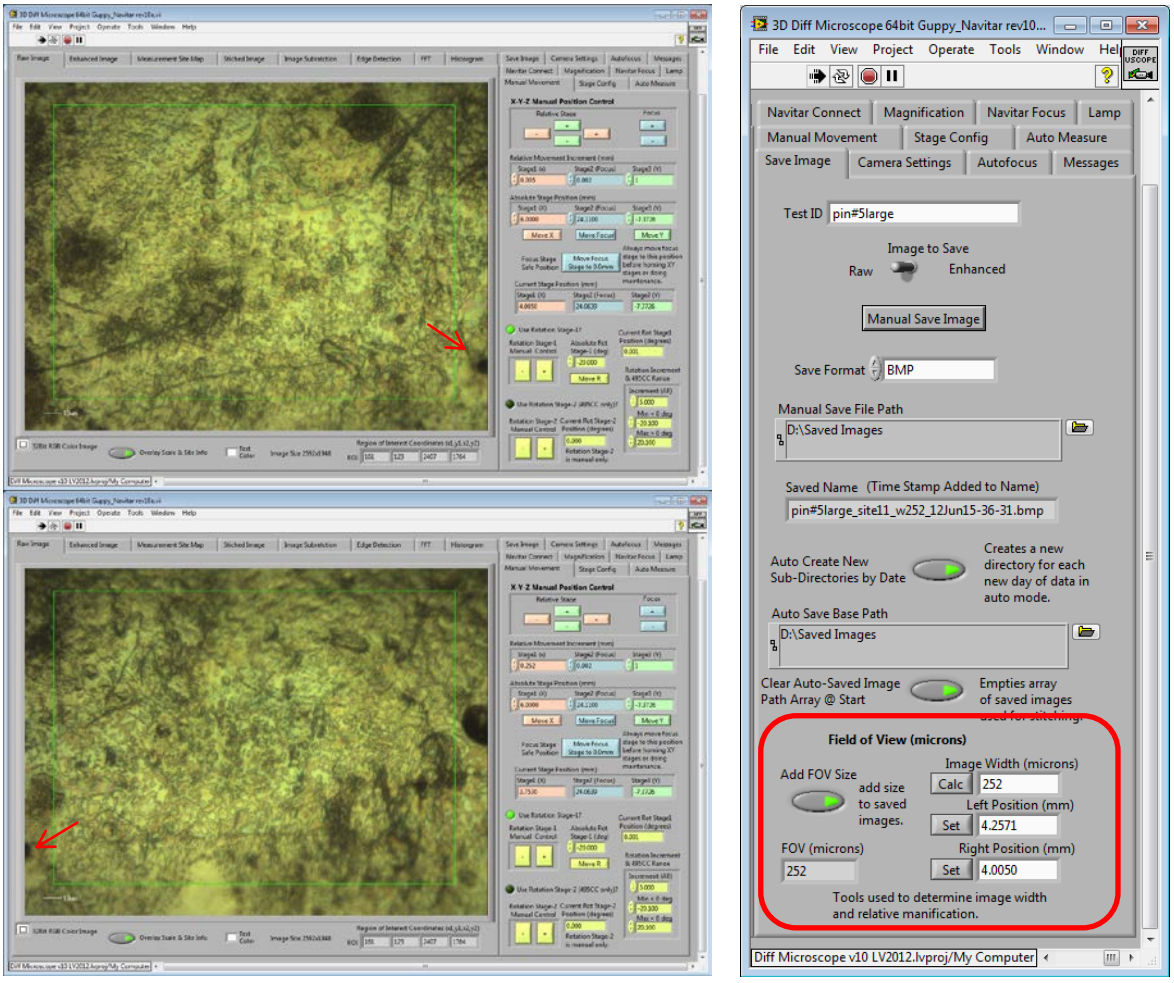


Figure D4. Image color values for a sample surface.

APPENDIX E – IMAGE SIZE MEASUREMENT

Image size measurement is important for determining microscope magnification and verifying the accuracy of defect size during analysis. At the bottom of the save image tab are controls for determining the image horizontal size in microns. To determine image size for a microscope magnification, the user navigates (using the manual stage controls) to a selected feature and positions it the left side of the image window and presses 'Set' left. The user then moves that same feature to the right side of the image window and presses 'Set' right. Image width is determined when the 'Calc' button is pressed. Figure E1 shows the screen views of each tab control during this measurement operation.



E1. Measuring Image Width / Field of View (FOV). Right position of feature (a). Left position of feature (b). Controls and indicators for image size measurement (circled in red).

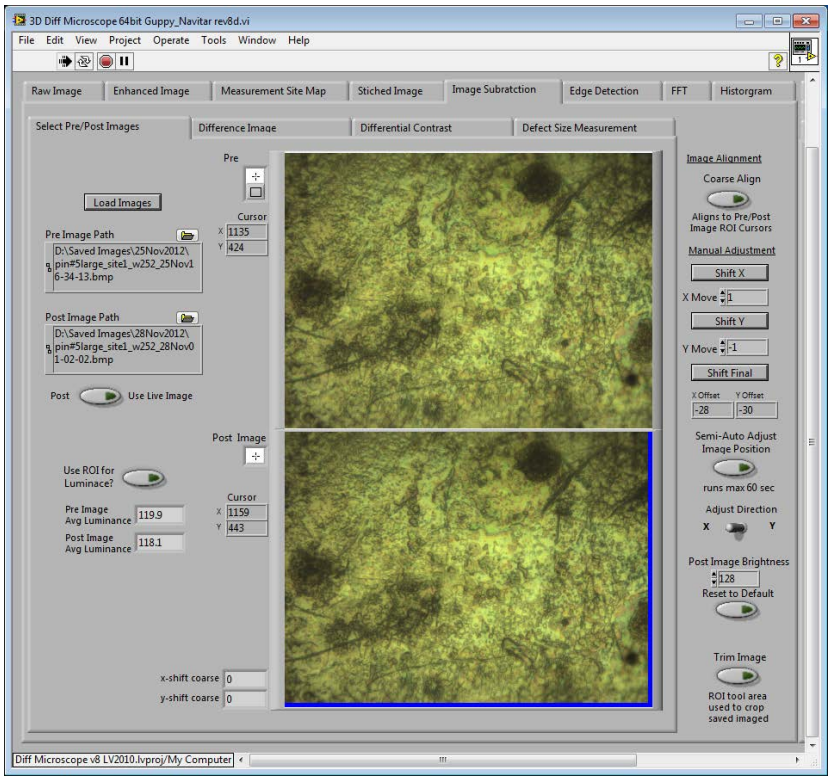
APPENDIX F – IMAGE POSITIONAL SHIFT MEASUREMENT

Comparison of images of the same site taken at different time periods is a method for determining the amount of sample and/or stage movement over time. If the image size has been accuracy determined from appendix E, the image analysis tab panels can be used to measure the change in image position versus time (Figure F1).

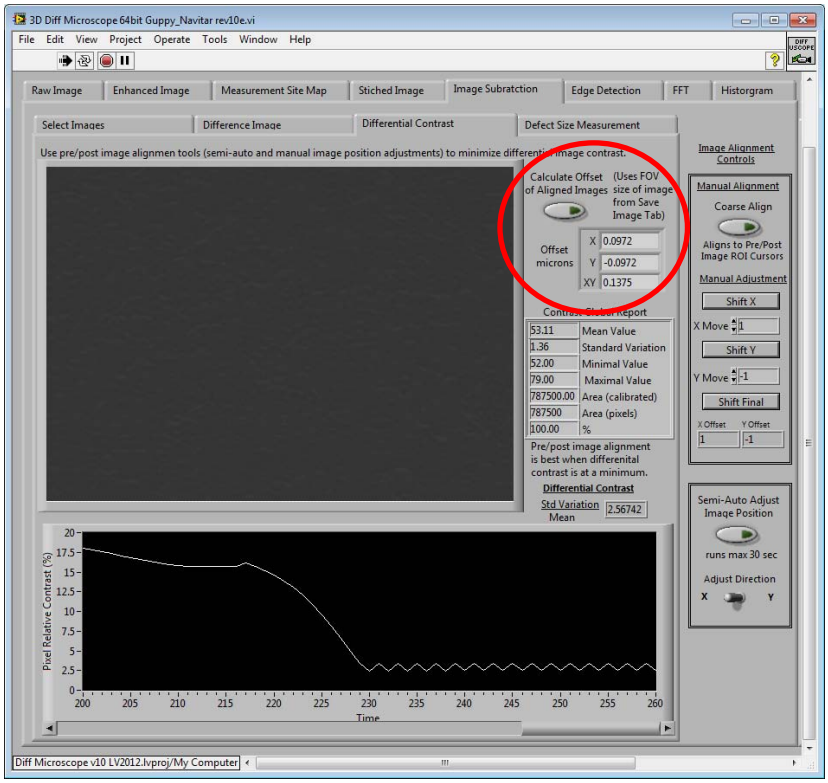
Only the pre/post image selection and differential contrast tab panels are needed for this measurement. To the right of these panels are a number of controls for aligning the pre and post images. The images for subtraction are selected with the path browser controls or the currently raw image viewed by Guppy camera selected as the post image instead by enabling the 'Post Use Live Image' button. The pre and post images may be coarse aligned by placing a crosshair cursor '+' on the same feature of both the pre and post images and pressing the 'Coarse Align' button. The pre and post images may also be matched for luminance (brightness) during subtraction over either the either image area or a selected ROI, using pre image ROI tool. The difference contrast tab panel includes a black & white view of the image area and is use for fine alignment to the pixel of the pre and post images. The difference image uses the resultant subtracted image of pre image RGB values less post image values. Negative difference values are clipped, setting them to 0. Typically, non-inverted or post image less pre image values are used. Optionally, inverted (pre image less post image) values may be used to display on the standard difference pixel values that are negative.

The difference image tab panel includes a black & white view of the image area used for alignment for either the entire image area or a rectangular ROI (that is selected from the difference image tab panel). The semi-auto adjust image controls are used to step the post image in either horizontal or vertical direction of X or Y movement increment in pixels to a position of minimal contrast alignment. Once the two images are aligned, a set of indicators is used for measuring the offset in microns between pre and post images. This offset distance is an important measurement to determine if significant image drift has occurred over time. Minimal image drift over an entire set of exposure images avoids having a large part of the subtracted image area be rendered useless. 5 microns drift per day is a maximum limit that should be allowed.

a



b



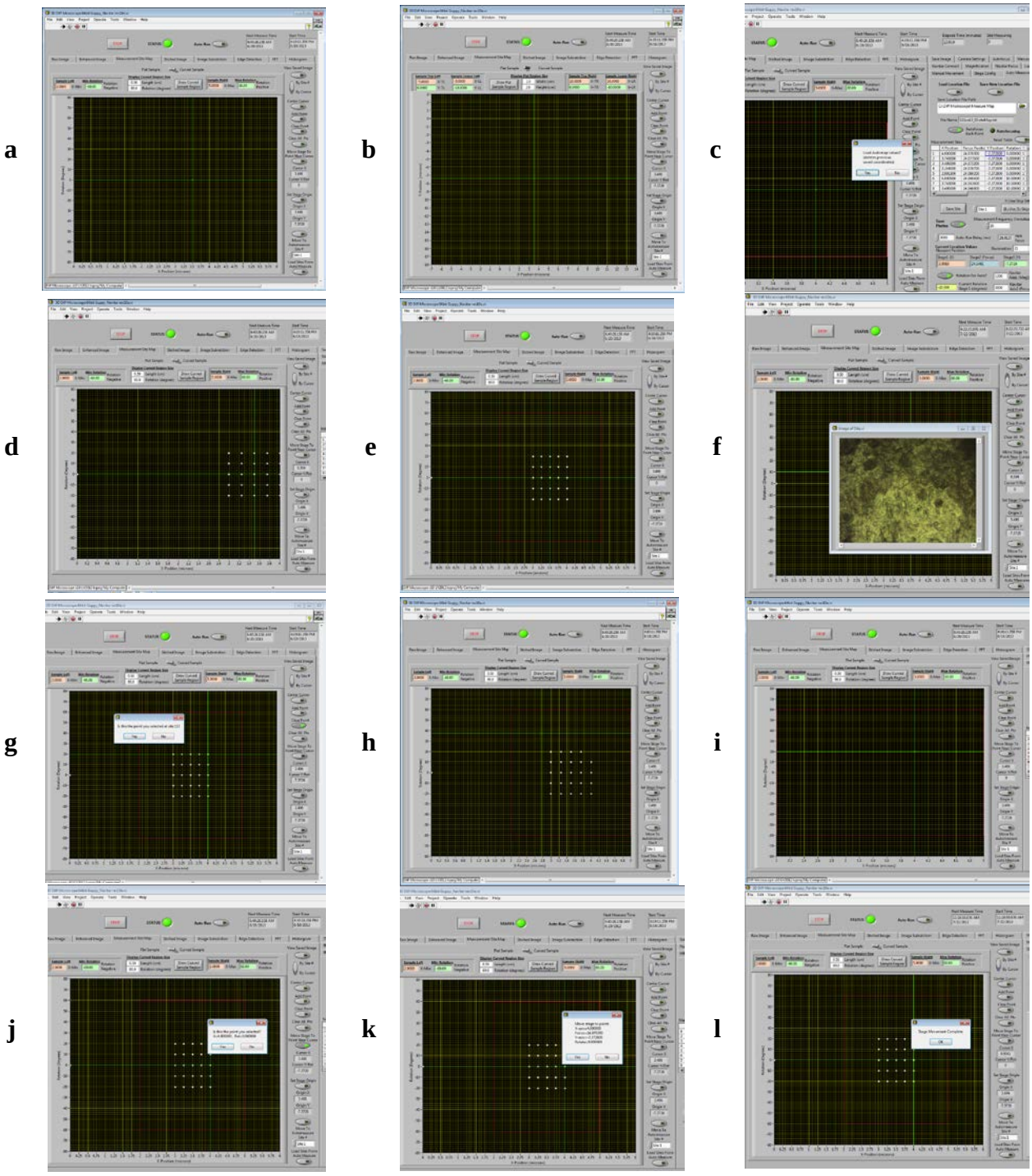
F1. Image alignment measurement. Selection of pre and post images (a) and image alignment and measurement (b). Measurement controls/indications are circled in red.

APPENDIX G – MEASUREMENT SITE MAPPING

The ability to map measurement size locations on an XY grid is a novel feature of the image acquisition software. This is an advanced feature of the software that should be used with caution, because a user could add points to the map grid that could potentially result in stage collision with the microscope objective. Screen captures of different view of the mapping tab control panel are shown in Figure G1.

Either curved pin samples or flat coupon samples may be viewed on a gird of horizontal x-axis position and either (a) rotation or (b) y-axis vertical positions. Movable vertical and horizontal green lines act as cursor position at the cross intersection. The software allows the user to either create a matrix of points on as starting points for measurement site locations or to populate the map with the values present in the measurement sites table (c and d). The sample center is set with by moving to stages to the middle of the sample and recording the center point value by setting stage origin. The sample size, map perimeter coordinates, and usable sample area may all be set by the user using input numeric controls at the top of the map area. The usable sample area is also displayed as a (e) red rectangle / rhombus. After a complete set of images have been acquired in auto-run mode, those images can be viewed (f) either by site number or cursor position.

Points may be manually added or deleted (g and h) from the map. The map may also be cleared of all points (i) by pressing the 'Clear All Points' button. Note that a single site is added to the map using the cursor position and pressing the 'Add Point' button, only the X-axis and Y-axis /rotation values are saved to the map. The only navigation method for that point is via 'Move Stage to Point Near Cursor' (j) where only the X-axis and either Y-axis (flat) or rotation (pin) stages are moved. The focus stage position is not altered. If the 'Move to Auto-measure Site #' is selected (k), then all stages (XYZ and rotation) are moved to the coordinates in the site measurement table. After movement is completed, a popup window appears (l) with the message 'Stage Movement Complete'.



G1. Measurement site mapping tab panel views and features. (a) Curved pin vs. (b) flat coupon sample grid configuration with cursor centered (b) on stage origin / sample center point (b). Load of measurement sites table (c and d) or auto-run coordinates. Drawing of curved/flat sample region (e) as red rectangle. Image of site selected (f) by either site number or cursor. Removing / clearing a point from the array (g and h). Clearing the entire array (i) of points. Move stages to site by cursor position (j) or site number (k). Movement complete (l) message.

APPENDIX H – NEWPORT ESP301 CONFIGURATION

Detailed information on operation of the ESP301 and stages is available in the *Newport ESP301 User's Manual.* Manuals are also available for each of the linear of rotation stages, if detailed information is each stage is needed. The system user should read and be familiar with the information in sections of 1 and 2 of the *Newport ESP301 User's Manual.*

The ESP301 front panel is shown in Figure H1. The LCD display shows the current menu item being viewed. Navigation and selection of menu items is through the 4 vertically stacked buttons immediately to the right the LCD display. Further to the left are jog button for moving stages manually. Note that stage 1 jog controls appear on the first line, stage 2 on the second line, and stage 3 on third. The power on/off switch is not shown and is on the read of the controller.

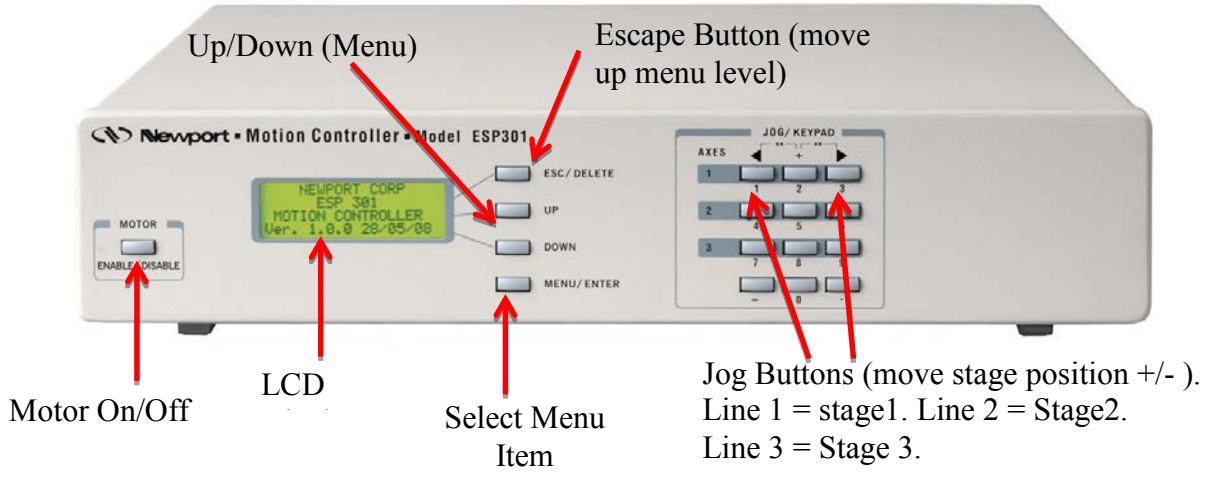


Figure H1. ESP301 Front Panel.

One of the most frequently used menu items is stage homing. Figure H2 shows the LCD panel displays for navigation to the stage home menu. Using the up/down buttons the selected stage (indicated by the diamond shape to left) may be homed. When homing XYZ stages, it is recommended that Axis 2 (focus position) be homed first to avoid possible collision of the exposure chamber with the microscope objective.

The XYZ-stage joystick is another option frequently used when adding or removing samples from the system. To avoid an accidental stage movement, the joystick is disabled during and after the LabView image acquisition is running. If the user wishes to use the joystick and y-stage controller, the XYZ stages should first be homed. The XYZ-stage ESP301 power switch is turned off and then turned back on, to re-enable the joystick. The joystick and vertical axis control will then be operational for an operator to move the XYZ stages manually.

a

b

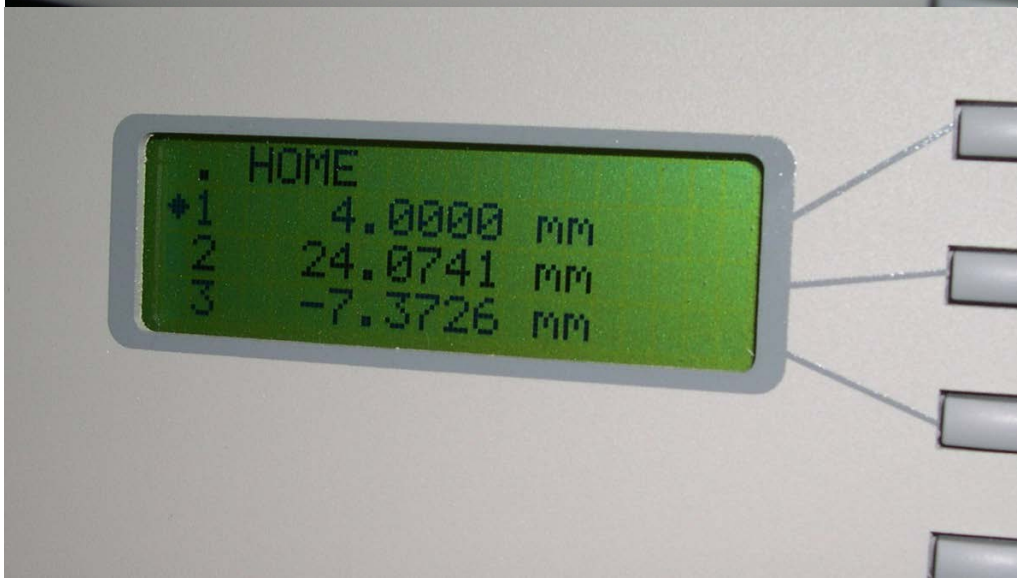


Figure H2. ESP301 LCD panel navigation from menu options (a) to home menu (b).

Additional LCD menu items include controller configuration and settings. Tables H1 and H2 show the configuration settings used for the ESP301 XYZ and rotation stage controllers.

Table H1. ESP301 XYZ Stage Settings

COMMUNICATION	IEEE CONFIG (GPIB 1)
STAGE MODELS	1) IL250CCHA 2) ILS50CCHA 3) VP-25XA
CONFIGURATION: SET VELOCITIES:LO JOG	1) 12.5 mm/s 2) 2.5 mm/s 3) 2.5 mm/s
CONFIGURATION: SET VELOCITIES: HI JOG	1) 50 mm/s 2) 10 mm/s 3) 5 mm/s
CONFIGURATION: SET VELOCITIES: HOME	1) 25 mm/s 2) 10 mm/s 3) 10 mm/s
CONFIGURATION: SET ACCEL	1) 200 mm/s2 2) 100 mm/s 3) 50 mm/s
CONFIGURATION: SET DECEL	1) 200 mm/s2 2) 100 mm/s 3) 50 mm/s

Table H2. ESP301 Rotation Stage Settings

COMMUNICATION	IEEE CONFIG (GPIB 2)
STAGE MODELS	1) SRC50CC
CONFIGURATION: SET VELOCITIES:LO JOG	1) 0.5 dg/s
CONFIGURATION: SET VELOCITIES: HI JOG	1) 2.0 dg/s
CONFIGURATION: SET VELOCITIES: HOME	1) 2.0 dg/s
CONFIGURATION: SET ACCEL	1) 8 dg/s
CONFIGURATION: SET DECEL	1) 8 dg/s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