ASET-LV4 User Guide

<u>Software Setup</u>

ASET-LV4 is a LabView application that is typically supplied with an installer that installs the following components and drivers needed to run the program:

- LabView 2012 Run Time engine [12.0.3, or f3]
- Measurement and Automation Explorer (NI-MAX) [5.3.1 / 4.6.2]
- NI-DAQmx [9.5.5 / 9.1.1]
- NI-IMAQ [4.7 / 4.4]
- NI-IMAQdx [4.7 / 3.6]
- NI-Motion [8.4 / 8.1]
- NI-VISA [5.2 / 4.6.2]

The exact version of the National Instruments hardware drivers can vary. The first version shown in the brackets above is the version typically deployed by the supplied installer, the second version is the oldest version for which operation has been confirmed. This is different from the protocol for operating ASET for Windows, where it was important to make sure a specific version of hardware drivers was installed.

The NI-IMAQdx functions used for interfacing with DirectShow compatible USB, FireWire, and GigE cameras, as well as certain vision functions used for difference image analysis, require a NI-Vision Run Time License (NI part 778044-35) to be installed. A license for one computer is provided with ASET-LV4. Licenses for additional installations must be acquired separately.

The ASET-LV4 program reads many settings from the file ASET-LV4.ini, which is stored in the same folder as the ASET-LV4.exe file. The folder containing the ASET-LV4.ini file must have read and write permissions for the User accounts that will be using the program. Otherwise the ASET-LV4 program will not be able to save new values to the ASET-LV4.ini file for the configuration and operation parameters that control how the program operates.

Hardware Setup

The following hardware is needed for typical operation of the program:

NI-DAQmx compatible A/D card (eg PCI-6221, PCI-6229, PCIe-6323) NI-Motion compatible motion control card (eg PCI-7334, PCI-7344) IMAQ compatible imaging device, for example either of the following : frame grabber (eg PCI-1411, PCI-1405, PCI-1424) IMAQdx license (NI part 778044-35) for USB, Firewire, or GigE camera

A typical device configuration tree in NI-MAX is as follows:

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There are two DAQ boards in the above device configuration. Only one board is needed, but more than one board can be accessed by ASET-LV4 to perform various functions. The details of the DAQ card channel selection and wiring configuration are handled by settings in ASET-LV4.

The NI Motion Device provides step and direction signals to the motor driver, typically a CMC-4 from Applicable Electronics, LLC. For the most demanding applications the NI Motion Device can be set up to use encoder feedback to ensure that the motor position tracks the programmed position. In many cases the NI Motion Device can be used effectively in Open Loop Stepper mode, which is specified by selecting the device in the NI-MAX Devices and Interfaces tree and selecting the Open Loop Stepper mode on the Current Settings page.

File Edit View Tools Help	tomation Explorer		
My System	😻 Initialize 🚯 Change Settin	gs 🖊 Save 🕼 Refresh 🖆 Defaults	💦 Hide Help
 Bata Neighborhood Bevices and Interfaces NI PCI-6229 "Dev1" 	Default 7340 S	Settings 1	GBack
 NI PCI-6221 "Dev2" Network Devices Image: MI Motion Devices 	Type of Settings: 7340 Settings Description: These are the initialize the mol	current initialization settings which are used to ion control device.	Use the Current Settings tab to configure the controller
 	Default Configurations: Open Loop Stepper:	Configures all axes for open loop stepper operation using factory defaults.	initialization settings. You can use MAX to configure the device to use default initialization settings or custom initialization settings.
▷ (m) Axis 3 ▷ (m) Axis 4 ▷ 20 Interactive ▷ 21 Calibration	Closed Loop Stepper:	Configures all axes for closed loop stepper operation using factory defaults.	The first time you launch MAX, the initialization settings appear in the configuration tree as Default xxxx Settings ,
 III-IMAQdx Devices J Serial & Parallel A Scales 	C Servo:	Configures axes for servo operation using factory defaults.	and all controller settings are pre- configured to factory default. Use the configuration tree items
 Software Semote Systems 	Custom:	Configure using custom settings.	Settings level to customize the controller settings.
			Caution Selecting one of the default configuration settings on this tab permanently changes settings for all axes on
	्रैङ्गि Current Settings 👯 Initializa	tion Preferences 🕹 Vector Spaces 😫 Sequence	

If a USB, FireWire, or GigE imaging device will be used then it is important to install the device manufacturer's DirectShow drivers before looking for the device in NI-MAX. For uEye cameras from IDS Imaging, the latest drivers can be downloaded from <u>http://www.ueyesetup.com</u>. The NI-IMAQdx device list will include any imaging device with a DirectShow driver. It is important to note the name of the device that will be used, which is cam0 in the example below:

Image: NI-IMAQdx Devices
Image: Cam0: UI164xLE-C_4002923174

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For some uEye cameras it is necessary to set the Vertical Mirror attribute. This is done as follows:

- 1. Open the NI-IMAQdx section of the Devices and Interfaces list
- 2. Selecting the imaging device (ie cam0)
- 3. Select 'All Attributes' in the View Options menu located just under the image window
- 4. Scroll down in the list of attributes until the 'Vertical Mirror' option is visible, and set it



The camera setup should be verified in NI-MAX at this point by clicking on the **Grab** button at the top of the middle pane of NI-MAX. An image should appear in the window, if it does not then the problem needs to be resolved before proceeding. Some older computers have trouble initializing the camera when the computer is first powered on. Updating the chipset drivers may solve the problem. In other cases, USB cameras with non-compliant drivers can corrupt the registry so the uEye camera will no longer be recognized.

Width

1280

A workaround that seems to provide consistent relief is to unplug the USB camera after starting the computer, wait 5 seconds, then reconnect the camera. This workaround should be done with NI-MAX and ASET-LV4 both closed. If that does not work, the following procedure should be tried:

- 1. Close NI-MAX and ASET-LV4
- 2. Unplug the uEye camera
- 3. Open Windows Explorer and search for *.icd on the C: drive, normally the uEye camera file will be found in the folder 'C:\Users\Public\Documents\National Instruments\NI-IMAQdx\Data'



- 4. Right click on the 'UI164xLE???.icd' file and select 'Open File Location'. Then select the 'UI164xLE???.icd' and 'cam0.iid' files and delete them.
- 5. Plug the uEye camera back in
- 6. Start NI-MAX and confirm that a live video image can be displayed

Once the hardware has been installed and recognized in NI-MAX, it may be necessary to change some hardware settings in ASET-LV4. This is accomplished by starting the program, navigating to the Hardware page, making the necessary adjustments to the settings, and closing ASET-LV4

using the **Exit Program** button. This will save the settings to the ASET-LV4.ini file so the hardware will be initialized properly next time the program is started. The basic settings on the Hardware page are described below.

The DAQ Setup page contains several pages that determine how the program acquires data from the sensors. There are three types of sensors presently supported:

- 1. Ion Selective Probe (ISP/PVP)
- 2. Scanning Vibrating Electrode Technique (SVET)
- 3. Optrode

There is a DAQ setup for each sensor type that provides a foundation for the Sensor setup (described in a separate secion) that is selected when an experiment is performed. The parameters specified in the Hardware setup include the sampling rate, input terminal configuration, and channels sampled.

rs Start	ISP AI Chan ISP AI.TermCfg ISP AO Chan
Calibration Senso	ISP Min AI ISP Min AO -10.00 -10.00 ISP Max AI ISP Max AO -10.00 -10.00
Chart Scan	wait samp/chan avg samp/chan baseline samp/chan 5000 5000 5000 ISP Rate 5000 5000
Log Plot Image	ISP DIO Port Sp Dev1/port1 Sp Dev1/port1 Sp Dio On ISP DIO Off Sp FF Sp Dio Off Sp Dio Off
Hardware	
Diagnostics	ISP/PVP SVET Optrode Optrode Monitor DAQ timeout 10.00 DAQ Window Visible DAQ Motion Imaging Other Printer Save INI Settings







Motion Board ID	tion Setup	x		Y		z		w
√j1 PID Rate	usteps per um	20.157	$\left(\frac{\lambda}{2}\right)$	20.157	(Å)	20.157	(À)	20.157
250 usec	Velocity (um/sec)	1000.00		1000.00	(Å)	1000.00	(À)	1000.00
	cceleration (um/sec/sec)	5000.00		5000.00		5000.00		5000.00
	eceleration (um/sec/sec)	5000.00		5000.00	(Ý)	5000.00	(À)	5000.00
	Direction (-1 or +1)	-1		1	(Å)	-1		1
	S Curve Intervals	100	(À)	100	$\left(\frac{\lambda}{2}\right)$	100	$\left(\frac{\lambda}{2}\right)$	100
n								
	Tip Setup	X		Y		z		W
	Tip Setup Velocity (um/sec)	X 100.00	() T	Y 100.00	Ê	Z 100.00	Ê	W 100.00
	Tip Setup Velocity (um/sec)	X 100.00 15.00		Υ 100.00 15.00	(÷) (÷)	Z 100.00 15.00		W 100.00 15.00
	Tip Setup Velocity (um/sec)	X 100.00 15.00 15.00	(†) (†) (†) (†)	Y 100.00 15.00 15.00		Z 100.00 15.00 15.00		W 100.00 15.00 15.00
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NI Sensapex ✓ Cursor Control On	Tip Setup Velocity (um/sec)	X 100.00 15.00 15.00 1000 0.00	- -	Y 100.00 15.00 15.00 1000 0.00	(Z 100.00 15.00 15.00 1000 0.00	() () () () () ()	W 100.00 15.00 15.00 1000

Sensapex Serial		tion Setup	х		Y		z		w
VISA resource	flow control	usteps per um	20.157	Ŕ	20.157	(20.157	(20.157
baud rate	timeout 500	Velocity (um/sec)	1000.00	TÂ)	1000.00	- ê	1000.00	T A)	1000.00
data bits (8)	termination char	cceleration (um/sec/sec) $\frac{x}{\tau}$	5000.00		5000.00		5000.00		5000.00
parity (0:none)	enable tchar	eceleration (um/sec/sec)	5000.00	() T	5000.00	(Å)	5000.00	()	5000.00
stop bits		Direction (-1 or +1)	-1		1		-1	(Å)	1
Sensapex Device	Sensapex Threshold (um)	S Curve Intervals	100	(Å T	100		100		100
🗍 1 Sensapex Axis Config	0.5 Sensapex Increment	Tip Setup	x		Y		z		w
XAxis 🖞 Z Axis	4 Sensaney Position Reads	Velocity (um/sec)	100.00	- ê	100.00		100.00		100.00
	Sensapex rosition neuros	1 V							
YAxis ZAxis Y Axis	2 Sensapex Max Retries	.cceleration (um/sec/sec)	15.00	(À)	15.00	$\left(\begin{array}{c} A \\ \hline U \end{array} \right)$	15.00	(Å)	15.00
$\begin{array}{c c} YAxis & \frac{Y}{V} & X Axis \\ ZAxis & \frac{Y}{V} & Y Axis \\ Angle & \frac{Y}{V} & 0 \end{array}$	2 Sensapex Max Retries	cceleration (um/sec/sec)	15.00 15.00	(†) (†)	15.00 15.00	÷	15.00 15.00	() ()	15.00 15.00
YAxis ZAxis Angle NI Sensapex	Sensapex Hoshidin (caus 2 Sensapex Max Retries 100	cceleration (um/sec/sec)	15.00 15.00 1000	(†) (†) (†) (†)	15.00 15.00 1000	÷	15.00 15.00 1000	() () ()	15.00 15.00 1000
VAxis 5 X Axis ZAxis 7 Axis Angle 0 NI Sensapex Cursor Control On	Sensapex Position reads	cceleration (um/sec/sec)	15.00 15.00 1000 0.00	() () () ()	15.00 15.00 1000 0.00	÷ ÷	15.00 15.00 1000 0.00	() () () ()	15.00 15.00 1000

Calibration Sensors Start	Camera MAQdx Cameras Camera 2 1 MAQdx Cameras Camera 2 1 Camera 2 1 Camera 2 Camera 2
Scan	Camera Setup
Chart	Lamp settle (msec)
Plot Image	PCI IMAQdx Img File Err Display Always on Top JPG Image Quality 95 JPG Image Quality Diff ms 0 4 source
Log	Image Save Interval (sec) 60 55
Hardware	Record ON Period (min) Record OFF Period (min) 0 0 0 0 0 0 0 0 0 0
nostics	Playback Polling Interval (msec) 🗍 50
Diag	DAQ Motion Imaging Other Printer Save INI Settings

Calibration Sensors Start	Cameral Interface jimg0 Camera2 Interface jimg1	IMAQ Error status code source Source Source Status code Source
Chart Scan C		Live Jamp Line Trig Lamp Live Lamp settle (msec)
stics Hardware Log Plot Image	PCI IMAQdx Display Always on Top Camera Polling Interval (msec) 200 Image Save Interval (sec) 60 Record ON Period (min) 1 Record OFF Period (min) 0 Playback Polling Interval (msec) 50	Grab ms JPG Image Quality ↓ 750 TIFF JPG BMP PNG Grab ms 0 0 5 5 5 5 5 5 5 5 5 5 5 5 5
Diagno:	DAQ Motion Imaging Other Printer	Save INI Settings

_

Sensors S	Temperature Serial VISA resource flow control I/2 COM1 I/2 None 0
Chart Scan Calibration	baud rate 79600 data bits (8) 77 77 7 7 7 7 7
Log Plot Image	Acquire Temp Temp Polling Interval (msec) $\frac{1}{2}$ 1000 Temp read delay (msec) $\frac{1}{2}$ 250
ignostics Hardware	

Start	Printers	
Sensors	Microsoft XPS Document Writer	
alibration		
Scan C		
e Chart		
ot Image		
Log PI		
Hardware		
jnostics		
Diag	DAQ Motion Imaging Other Printer	Save INI Settings

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Exit Program

After reviewing all the above settings, click	
written to the ASET-LV4.ini file.	

and the settings will be

Then restart ASER-LV4 and begin the final step of the hardware setup, which is to define the Optical Scales that will be used. Scales can be added or edited at any point during an experiment, but it is critical to have the correct scale defined and selected when running the program. Otherwise any program operations that show measurements superimposed on the image will be incorrect, and any operations that involve using a cursor to show a position on the image will be incorrect.

Defining Optical Scales

The Optical Scale defines the relationship between screen coordinates and position coordinates. The makes it possible to display sensor data as an overlay on top of images. It also enables use of the cursor to show the program position in an image, for example where a calibration source is located, where to make a reference measurement, where to start and end a scan, etc.

Before defining an optical scale, it is strongly recommended that the camera and motion system axes be aligned to a reasonable level of agreement. The simplest method for doing this is to move the sensor to an edge of the image, watching the distance from the edge of the sensor to the edge of the image. This can be done by manually rotating the knobs on the motors to position the sensor, or by using the cursor control keys to move the sensor. The probe can be moved around using the cursor keys:

<left> / <right></right></left>	X axis
<up>/<down></down></up>	Y axis
<home> / <end></end></home>	Z axis
<pg up $>$ / $<$ pg dn $>$	W axis (focus)

Unless the Cursor Control On option is selected (Hardware>>Motion page) it is necessary to hold the Ctrl key when pressing the cursor keys. This is a safety measure to prevent unexpected moves of the probe. Note that the main ASET-LV4 program window must be the top level focus of the operating system in order to have the key presses processed.

If the distance from the edge of the sensor to the edge of the image remains the same across the full length of the image, then the systems are aligned. If the distance grows or shrinks steadily, then the camera should be rotated to correct the alignment. The example below shows a system that is not quite aligner properly, the camera should be rotated counter-clockwise to correct the alignment. Always repeat the alignment test after making a correction.

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If the distance grows and then shrinks, there is usually geometric aberration in the imaging system. Geometric aberration is not desirable, but can be acceptable. If geometric aberration is present, the distance from the sensor to the edge of the image should be symmetric about the center of the image, and the optical scale definition procedure should be accomplished over a region that is centered about the center of the image.

Navigate to the Image>>Overlay page:

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Difference Overlav Image	Difference Overlage Image	Vector P $\frac{1}{7}$ 1 $\frac{1}{7}$ Solid Pix/sc $\frac{1}{7}$ 50 Overla	en ale unit Head Ang 15 ay Text	le Head Pixe	Vector Da secor 9:06: 9/17 els	ata nds since 1Ja 54.937 PM /2012 Horizontal 499 Vertical 422	Horizontal 499 Vertical 422	Horizontal 499 Vertical 422	Horizontal 499 Vertical 422	Horizontal 499 Vertical 422	Horizontal 0 Vertical 0	Horizontal 0 Vertical 0
[msca Timer	image limes		Scale Name New Scale Optical Scales	Define I	New Optical	Scale	OSD 3 OSD 1 512	Step P1 Coords 389	OSD P	Pos1	0	
TVA edeby	VIAKE AVI	- D	Name Default Name	x um/pix 1.49573	y um/pix -1.50602	rotation 0	OSD I	P2 Coords 389		ο γ	0	
			4x Name	11.2676 x um/pix	-11.0345 y um/pix	0 rotation	512	389	X	0 Y	0	
			Max Mag Name Default	1.50377 x um/pix	y um/pix	o rotation	₽	Pixels 50 os Scale Pos	⊽ Grad	d1 Scale Pos	Grad2	Scale Pos
			Name Default	x um/pix	y um/pix	rotation 0	Let	ft Top 50 🔂 400	Left	Тор 7) 30	Left 7 550	Top 7 30

Scale Name

Enter the name of the scale to create or edit in the New Scale field. To edit an existing scale, make sure the case and spelling match the scale that is being edited. If the existing scale name is not visible, the Optical Scales spin control can be used to make it visible.



Click on the Define New Optical Scale button. This will start the optical scale definition process. It is important to complete the entire process before using other features of the program, otherwise the optical scale will not be correctly defined. Focus will shift to the Image>>Image page, a cursor crosshair will be displayed, and the message 'Show probe in TOP LEFT' will be displayed.

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tion Sensors Start	Overlay Image	Show p	robe in TOP LE	IFT							Grad1 Scale
Image Chart Scan Calibra	ike AVI 🍵 Image Times 🍵 Difference										Optical System New Scale Show Sensor1 2147483 -214748.
Diagnostics Hardware Log Plot	Ma	Last Image	15:12:04.044 3/12/2013	Image Seek	18:58:17.996 9/13/2012	Record Start	22:09:24.603 1/26/2010	Record End	22:10:24.603 1/26/2010	Recording Images	Concentration Pos Left Top 107 43 Gradient Pos Left Top 107 47 Text Color Persist (sec)

Complete the following procedure:

- 1. Use the cursor control keys to move the probe close to the top left corner of the image
- 2. Position the cursor over the probe, and left-click once.
- 3. The message 'Show probe in TOP RIGHT' will be displayed. Use the cursor control keys to move the probe along the X axis only until it is close to the top right corner of the image.
- 4. Position the cursor over the probe, and left-click once.
- 5. The message 'Show probe in BOTTOM RIGHT' will be displayed. Use the cursor control keys to move the probe along the Y axis only until it is close to the bottom right corner of the image
- 6. Position the cursor over the probe, and left-click once.

After this the message 'OSD Complete!' will be displayed. The new values will be updated in the display and saved to the ASET-LV4.ini file.

Click the Show Sensor1 button, position the cursor over the probe, and left-click once. The x 0.0

y 0.0 position coordinates displayed in the main program window will be updated with the

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new position of the probe. This is a good time to click the **OZ** and **OW** buttons to zero the

- Z 0.0
- W 0.0

position coordinates displayed in the main program window.

Program Operation

When the program starts, three video windows and the DAQ Display window should open. These windows may need to be positioned in different places on the desktop to allow viewing the main program window.



The 'ASET-LV4 Image' window is updated regularly with an image acquired from the camera specified in the Hardware setup. The 'ASET-LV4 Reference Image' window is updated when the 'Get Ref Image' button on the Image>>Difference page. During acquisition, the 'ASET-LV4 Difference Image' window is updated every time the 'ASET-LV4 Image' is updated. More details about the acquisition, display, and storage of difference images can be found in the Difference Imaging section.

The DAQ Display window shows analog input data from the data acquisition boards when acquisition is in progress. The display is intended to provide a direct view of the analog input data before it is processed into values relating to the sensors being used.

The Context Help window contains many notes explaining features of the program. The window updates when the cursor is moved over a new control for which there is documentation available.

The first screen for ASET-LV4 requires a choice of one of four modes:



Start Single Sensor

and a dialog will appear asking where to store the files:

ASET Specify Data Filename					X
Solution ASET-LV	4 Data 🔸 Sample Data		Search Sam	ple Data	P
Organize 🔻 New fold	ler			•= •	0
 ★ Favorites ▲ Desktop ▲ Downloads ▲ Recent Places ■ Libraries ▲ Documents ▲ Music ▲ Pictures ▲ Videos 	Name	Date modified No items match your search	Туре	Size	
🚜 Homearoup 🔻					
File n	aame: ASET-LV4 Data 2013-03-12 18-57-01.	csv	Custom Patte OK	ern (*.csv) Cancel	•

It is recommended to select a root folder (eg 'Sample Data' in the dialog above) that is unique to the experiments being performed. Avoid storing lots of experiments in the same root folder.

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It is also recommended to keep the date and time in the filename as this can help find data files during the review process. Sometimes the File name field does not display the full name.

File name:	12 18-57-01.csv	-
------------	-----------------	---

Setting the cursor down in the File name field will cause the full name to be displayed.

For each session, the program creates two comma-delimited text files and three subfolders. One comma-delimited text file stores data and the other stores manual and automatic log entries (calibrations, references, image times). One folder stores images, one stores scans, and the third stores analysis data (primarily for difference viewer operations).

Name	Date modified	Туре	Size
퉬 ASET-LV4 Data 2013-03-09 19-12-39 Analysis	3/9/2013 7:12 PM	File folder	
퉬 ASET-LV4 Data 2013-03-09 19-12-39 Images	3/9/2013 7:12 PM	File folder	
퉬 ASET-LV4 Data 2013-03-09 19-12-39 Scans	3/9/2013 7:12 PM	File folder	
ASET-LV4 Data 2013-03-09 19-12-39.csv	3/9/2013 7:12 PM	CSV File	1 KB
ASET-LV4 Data 2013-03-09 19-12-39_log.csv	3/9/2013 7:12 PM	CSV File	1 KB

The default mode of the program is to store moderate quality video images every few seconds

(see Image Save Interval (sec) 60 on the Hardware>>Imaging page). These images can be played back in the review mode and used to navigate through the probe signal dataset. The images must be stored in a subfolder with the same name as the filename root, changing the folder name or the name of any of the image files will interfere with the operation of the review environment. Likewise changing the data stored in the .csv or _log.csv files may also interfere with the operation of the review environment.

When modifying any images or files for data analysis and presentation, it is best to make a copy of the original files and modify only the copies, not the originals.

Once the root folder is selected and the filename is specified, the following screen should appear:

Start	Sensor 1 Name 💮 pH Probe Sensor 2 Name 💮 2D SVET	2 Sensors
Scan Calibration Sensors	ISP1 Setup D/A chan A/D lo chan A/D lo gain A/D hi chan A/D hi gain 0 1 10 0 100 Ion1 Calibration Slope Intercept Efficiency D cm^2-s 29.1 0.1 0.8 9.37E-5 Good Slope Slope +/-	
ot Image Chart	29 5 wait sec avg sec base sec cal sec 0.5 0.5 0.5 3 User Direct A/D SVET ISP Optrode Ext	
cs Hardware Log Plo	S1 min wait S1 min avg S1 pH Grad1 Grad1RC Grad1RI Grad1RO X Y Z 0.5 0.5 ISP pH Probe 0 1 0 <t< td=""><td></td></t<>	
Diagnostic	Wait sec Sample sec Baseline sec Polling sec Polling = time between end of one sample and beginning of next 0.5 0.5 0.5 0.5 0.02 0.02 Continue	

This screen is for choosing the sensor type and specifying the sampling rules that will be used.

The sensor is selected with the ^{Sensor 1 Name Ca2+ Probe} control at the top of the page. The D/A and A/D settings are read-only at this point in the program. The type of the sensor is automatically determined by an entry in the ASET-LV4.ini file. To edit these values, use the **Exit Program** button to leave the ASET-LV4 program, edit the corresponding values in the ASET-LV4.ini file (see Appendix <u>A/D Channel Setup</u>), and then restart the ASET-LV4 program.

The sampling timing is defined by the following controls:



wait sec $\frac{\lambda}{\tau}$ 0.5

0.5 is the amount of time that the program pauses, after moving the probe to a sampling position, before it starts using A/D data.



Rotation

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avg sec

is the period of time during which that the program acquires data from the probe after the wait cycle.



^{0.5} is the period of time during which that the program acquires data to establish the baseline for the ion selective probe post-amplifier. This sampling occurs prior to the wait cycle, only when enabled and only when the sensor has an Ion Selective Probe type.

The sampling path is set by the following control:

Tip pt	:s (um)	I			
	Х		Y	Ζ	
(À)	10		0	0	
(A)	0		0	0	
) E	0		0	0	
A)	0		0	0	

The first point in the sampling path is the origin, with index 0. The next point, with index 1, is the first point in the 'Tip pts (um)' list. In the above example, there is one point at the position X=10 microns, which will be 10 microns to the right of the probe position at the start of the sampling cycle.

Note that this sampling path can be rotated by specifying a non-zero value in the o control at the lower right of the main program window. This control specifies a rotation in degrees counter-clockwise. For example a Rotation of 90 will result in the second point (at index 1) that is 10 microns along the positive Y axis from the probe position at the start of the sampling cycle.

There are two calculations available for real-time viewing of the data that is being acquired by the program. The data at each sampling point is stored regardless of how many points are in the 'Tip pts (um)' list, but only two gradient calculations are made and stored during acquisition.

_Grad1	Grad1RC	Grad1R1 Grad1R0
Ca2+ Probe		$\left(\frac{\lambda}{\tau}\right) = 1 - \frac{\lambda}{\tau} = 0$
Grad2	Grad2RC	Grad2R1 Grad2R0
Ca2+ Probe	0	÷ 0 - ÷ 0

A wide variety of display combinations are possible.



Grad1		
<u>(</u>)	Ca2+ Probe	
ž	Grad2	
$\left(\frac{\Lambda}{2}\right)$	Ca2+ Probe	

determines which sensor's data is selected (only one option is valid for single sensor experiments)

For the following fields, index 0 is at the 'Origin' (the probe position at the start of the sampling cycle), index 1 is at the first point in the 'Tip pts (um)' list, etc.



indicate the indexes of the samples that are used to calculate the concentrations that are displayed for the selected sensors.



indicate the indexes of the samples that are used as minuends to calculate the gradients that are displayed.



indicate the indexes of the samples that are used as subtrahends to calculate the gradients that are displayed.

	Continue
After adjusting the above settings, click	



The Calibration page will appear:

The program will start reading calibration data from the A/D card and displaying the samples in the DAQ Display window. The data from the channels associated with the probe being calibrated will be displayed as voltages in the mini-display at the bottom of the calibration window. The average of the DAQ samples shown in the mini-display will be used to calculate

	0.582		44.05	1.834	
the	Tip uV	and	Nernst mV	readings shown at the top of the display (or Magnitude and	
58	.19				

Degrees for oxygen probes).

To calibrate the probe, enter the calibration solution concentrations in the C1, C2, and C3 fields (in mM for ion selective probes, or in % for oxygen probes):

Sensor1 C1 分	10
Sensor1 C2 分	100
Sensor1 C3	100

Place the sensor successively in the calibration solutions. For each solution, wait until the signal in the mini-display stabilizes, then use one of the following buttons to apply the most recently displayed data to the calibration calculation for the associated solution:

Acquire C1 Acquire C2 Acquire C3
0.582 58.19 The Tiputy (or Degrees) reading will be conied to the calibration table and the sampled
The field (of beginss) reading will be copied to the canoration table and the sampled
indicator will turn on <i>solution</i> . Note that a 3-point calibration will only change the
intercept, not the slope, for Least Square
After making the required measurements, click Continue. If the calibration is complete, a dialog will ask
Remember calibration for next session? Yes
Selecting Yes will saving the calibration values to the .ini file, allowing them to be used during the next session without performing a new calibration. To use the values stored in the .ini
file, skip the calibration steps described above and just click Continue to being
acquiring data. In that case, or if the calibration is not complete, a dialog will ask

2
New calibration is not complete.
Continue with last remembered calibration?
Yes No

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In either case the calibration values used with the experiment will be stored in the _log file. Then the Chart display will appear:



The time axis displays the time relative to T0, which is determined as the time when the calibration is completed and sampling begins. Every sample is continuously recorded in the .csv data file, both the concentration (in mM or %) at each point (calculated by applying the calibration to the measured voltage) as well as the two gradients defined on the Sensors tab.

There are cursors to aid with inspecting values on the chart, and to establish a 'Reference' or 'Background' value. Clicking on the center buttons will bring the associated cursor to the middle of the current chart display. The cursor can then be adjusted by selecting the Cursor tool

The Time Span (min) 3 control determines how much time is displayed on the chart. Unchecking this control makes it possible to use the horizontal scrollbar or the chart controls



to modify the chart display. Many aspects of the chart can be controlled by right clicking on various parts of the chart. New values for the scales can also be typed in directly, although it is necessary to turn off the autoscale feature for the settings to be preserved.

The probe can be moved around using the cursor keys:

<left> / <right></right></left>	X axis
<up>/<down></down></up>	Y axis
<home> / <end></end></home>	Z axis
< pg up > / < pg dn >	W axis (focus)

Unless the Cursor Control On option is selected (Hardware>>Motion page) it is necessary to hold the Ctrl key when pressing the cursor keys. This is a safety measure to prevent unexpected moves of the probe. Note that the main ASET-LV4 program window must be the top level focus of the operating system in order to have the key presses processed.

um/keyhit

The move increment for each key press is set by the 9^{100} field on the right side of the program window. The move increment can be doubled by pressing the '+' key on the numeric keypad or halved by pressing the '-' key on the numeric keypad. If the Shift key is held while pressing a cursor control key, a move that is 10 times the current move increment will be made.

It is important to avoid excessively large moves that may cause the sensor to crash, and it is also important to avoid loading up the keyboard buffer with many keystrokes in order to get the um/keyhit

um/keyhi

sensor to move a long distance. First set the $\sqrt[9]{100}$ to a suitable value, then use only a few slow keystrokes to move the sensor to the desired position.

Note that data will be acquired and displayed in the program window, but it will only be

recorded to the disk when the red Record button 🖤 is enabled.

The Image display can also be used to view the absolute concentration as well as the gradient graphs superimposed on the current image.



The position of the top left corner of the overlaid charts can be adjusted using the Conc Pos Flux Pos

Left	Тор	Left	Тор
107	45	and () 107	253

and and controls. Specifying a position beyond the right edge of the display window will effectively remove the chart from the overlay. The color of the plots is

set by the colorboxes and and

Optical System

Optical System

The ^{34x} selection determines the relationship between positions shown on the image window (for example using the ^{5how Sensor1} button) and physical positions of the sensors. It is important to have the Optical System properly defined and selected when defining grid scan positions. Showing the position of the sensor in the image also sets the position stored



Persist (sec)

with each entry in the data file. Overlay vectors from the past ¹⁰ samples are displayed on the image at a location determined by the probe position and the selected Optical System.

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<u>Scans</u>

Before starting a scan, it is best to make sure the program knows the location of the sensor(s) and the optical scale that is being used. If the optical scale has not yet been defined, please define it before trying to scan. Instructions are provided in the Optical Scales section.

When it is certain that the optical scale is properly defined, complete the following proceudre:

- Navigate to the Image>>Image page. This can be accomplished using the tab controls on the left side of the program window, or by clicking the Image button at the top right of the program window.
- 2. Select the correct optical scale using the 2x control in the middle of the page.
- 3. Click the **OZ** and **OW** buttons to zero the **W O.O** position coordinates displayed in the main program window.
- 4. Click on the Show Sensor1 button
- 5. Position the cursor over the probe, and left-click once.

χ 0.0

The γ 0.0 position coordinates displayed in the main program window will now show the current position of the probe. Click on the Scan button or go to the Scan page to set up a scan.



Select the Line, Grid, or Vector scan tab as appropriate. For Grid scans, the X axis is incremented most frequently, followed by the Y axis, Z axis, then W axis. Only a flyback pattern is presently supported.

The Scan Home is the position to which the probe will return (if enabled) at the end of each scan.

The Ref Before and Ref After positions are the locations at which reference measurements will be made, if enabled, either before and/or after a scan is performed.

An image can be stored at the beginning and/or the end of each scan. This is independent of the top level periodic cycle that store an image at a regular interval.

Confirm that the 4x is correct. The program automatically synchronizes this control with the 4x control on the Image>>Image page.

Either type in the desired coordinates for the scan operations, or use the Show buttons to show the locations of the scan definition on the screen (this only works if the optical system is correctly defined). The scan will be performed with the sensor definition that was selected at the

program startup, using the sampling rules on the Sensors tab. The estimated scan time will be displayed in the bottom right of the Scan page. It is important to make sure there is enough time for each scan repetition and scan interval to complete. Note that the intervals are from start to start, so 30 seconds means there will be 30 seconds between the first observation of the first scan and the first observation of the second scan. A 'Schedule' indicates how many times to perform each set of 'Repetitions'. With the values above, there would be 3 sets of 6 scans. Each of the 6 scans would be started 30 seconds apart, and each set of 6 scans would start 20 minutes apart.

After defi	ning the scan parame	ters, use the Save Scan Definition	button to store the parameters
for future	use. Then click the	Start Schedule	button to begin scanning.
A scan car	n be interrupted by cl	licking on the Cancel Scan bu	utton located just under the status
indicator	Acquiring Sample 2	at the lower right corner of the i	main program window. Be

patient with the program while it responds, the scan will not be able to cancel properly until the most recent sensor sampling operation completes, which can take many seconds.

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<u>Plotting</u>

During a scan, the data will be displayed on a 2D and 3D plot. These plots can be viewed on the Plot tab, or by clicking on the Plot button and then selecting the 2D or 3D page.



After a scan is complete, the data can be reloaded by going to the Plot Data tab. Select an image by clicking on the folder icon at the right of the Image File control at the top of the page:

Image File C:\Users\Eric Karplus\Documents_program\ASET-LV4\Sample Data\11x11 scans\ASET-LV4 Data 2013-01-20 10-41-20 Images\ Image_2013-01-20_10-47-26.758.jpg

Select the scan folder by clicking on the folder icon at the right of the Scan Data Folder control at the top of the page:

Scan Data & C:\Users\Eric Karplus\Documents_program\ASET-LV4\Sample Data\ASET-LV4 Data 2013-02-06 14-11-44 Scans Folder the data file.

All the scan files will be loaded into the list. Note the

values

Data Index

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determine what value is plotted, normally these values should indicate the same quantity from

Z Index

XInPh

Double click on a scan file in the list to load it into the plot. Hold shift and double click on a file to change whether it will be included in the plot sequence. Click on Make Sequence to generate all the plots, then go to the Plot Sequence page to browse the results. 50 Image C:\Users\Eric Karplus\Documents_program\ASET-LV4\Sample Data\11x11 scans\ASET-LV4 Data 2013-01-20 10-41-20 Images\ ⁸ Image_2013-01-20_10-47-26.758.jpg File B Scan C:\Users\Eric Karplus\Documents\ program\ASET-LV4\Sample Data\ASET-LV4 Data 2013-02-06 14-11-44 Scans Data Sequence Folder Timestamp Scan Name In Seq A Plot Settings Plot Data Ŧ ✓ Autoscale plots after loading Make Sequence Header Rows Col Label Line X Index Y Index Z Index Data Index Vector X Vector Y <u>x (*</u> <u>^)</u>0 () Y 🖯 XInPh 🖯 XInPh XInPh 1 🗄 YInPh 0-20-40 E 60-80 -0.00 0.00 0.00 0.00 0.00 0.00 0.00 100 -0.00 0.00 0.00 0.00 121 -5 6 8 9 10 16 17 18 11 12 13 14 15 19

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Difference Viewer Technique

Basic functions of the former DVIT software are incorporated in ASET-LV4. To begin a DVIT

Start Difference Images

mode only experiment, click on the button on the opening page of the program window. If sensor data is to be acquired along with difference image mode, then proceed with the sensor acquisition instructions above. The DVIT functionality will be available at the same time that the sensor measurements are being made. Note that when sensor measurements are being made, there system may not be able to rapidly acquire and display the image data.

The ASET-LV4 program maintains a near-real time display window. The default update rate for

this window is set using the ^{Camera Polling Interval (msec)} field on the Hardware>>Imaging tab. The default interval is set to 1000 milliseconds, which may seem a bit slow for focusing operations. It is recommended to keep this interval as long as possible to minimize the amount of time needed by the system to process image data. The difference displays are updated for every 'camera polling' image that is acquired, but the images are only stored at the interval

specified by the Image Save Interval (sec) $\frac{1}{2}$ 60 control. Note that images will only be saved to

disk if the red Record button vision. The camera polling interval should allow for at least one image update for every image save interval, which is the amount of time between stored images.

When sensor data is being acquired, it is not recommended to set the camera polling interval below 250 milliseconds. When sensor data is being collected, it is possible to save an image corresponding to the beginning of each sampling cycle. This occurs when the **Img** control is Sampling Interval (sec)

turned on (located next to the 1.12 control at the lower right of the main program window). These images are used to update the difference image displays.

There are three image windows displayed for the DVIT technique: Image, Reference, Difference. The presence and size of these windows is controlled by the following controls on the Image>>Difference tab:



A positive zoom magnifies while a negative zoom demagnifies the image (ie -2 means half size, -3 means a third the size, etc.). The zooms -1, 0, 1 are all 'full size'.

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The normal operation of the program is to display a difference image and difference data at the Camera Polling Interval (msec) 1000 and record images at the image save interval Image Save Interval (sec) 60 . In analysis mode it is only possible to generate difference images using stored images.



Show Diff ROI

The program allows an ROI to be defined in the Image window using the button on the Image >>Difference tab. Using this button shifts the focus of the program to the Image window, and it is necessary to click OK or Cancel in that window to continue.



The data from the selected ROI is used to generate histogram, chart, and graph displays associated with the difference image data. The chart and graph display a 'histogram shift' value, Diff Offset

which is calculated as the weighted difference of the histogram value from the value, normalized by the number of pixels in the ROI. For example, if Diff Offset is 128 and the histogram has 20 pixels at 126, 70 pixels at 127, 100 pixels at 128, 25 pixels at 129, and 11 pixels at 130, the histogram shift would be (20*|126-128|+70*|127-128|+25*|129-128|+11*||130-128|) / (20+70+100+25+11) = (40+70+25+22) / 226 = 0.69.



The Chart displays the R,G,B histogram shift values for each image acquired at the Camera Polling Interval. The chart will hold up to 1024 data points, which can be displayed by changing the X axis values (place the cursor on top of the label, type in the new value and press Enter).



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The Graph displays the R,G,B histogram shift values for each image stored at the Image Save Interval. The Export Shift Graph button can be used to export the data in the graph to a text file for external analysis.

The Save Difference Image button can be used at any time to store the difference image visible at the time the button is pressed. The difference image is stored in the Analysis subfolder to avoid interfering with the structure of the Images subfolder, which holds only the images acquired during the experiment.



<u>Analysis</u>

Print Panel

It is possible to print a copy of the view on the screen at any time using the

button. The printer is selected on the Hardware>>Printer tab.

The data is stored in comma delimited text files with the .csv extension.



<filename>.csv - contains the measurement times, positions, and data values <filename> log.csv - contains any user comments as well as calibration data

	9),	SET-LV2 D	ata 2010-0	5-10 13-2	29-31.xls										
I		A	В	С	D	E	F	G	Н		J	K	L	M	
I	1	Timestamp	Time (sec)	Х	γ	Z	W	dR	dP	Angle	C1	C2	J1	J2	
I	2	40308.56	65.578	0	0	0	0	10	20	0	5.25637	0	2.876492	0	
I	3	40308.56	69.578	0	0	0	0	10	20	0	11.8253	0	2.524823	0	
	4	40308.56	73.578	0	0	0	0	10	20	0	19.06519	0	2.610708	0	

These files can be opened in Excel, but it is important to save any changes in a new file and not overwrite the original file. This is because the ASET-LV4 program depends on the original format of the file being retained for analysis operations.

Review Data

button on

To reload a file for analysis, start the program and click on the the opening page. Select the <filename>.csv file and the program will load the data and also inspect the Images subfolder for any images that were acquired.

The Chart and Image tabs can be used to view the data.





The slider at the bottom of the main program window can be used to browse the data or images.



Dragging and releasing the slider at any given time will pan the center time on the concentration and gradient graphs on both the Chart and Image tabs.

The Play Video button will automatically advance the image time slider from its current position Play Speed times real time. The << REW button

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will move the slider to the beginning of the recorded images. This will clear the histogram shift data from the chart and graph.

The Set Ref Image button can be used to set the presently displayed Image to the Reference Image. Subsequent updates of the Difference Image and histogram shift data will make use of the new Reference Image. Previous histogram shift data will always refer only to the data as defined by the Reference Image selected at the time the calculations were made.

As during acquisition, the Save Difference Image button can be used at any time to store the difference image visible at the time the button is pressed. The difference image is stored in the Analysis subfolder to avoid interfering with the structure of the Images subfolder, which holds only the images acquired during the experiment.

Appendix - uEye Camera Setup

For the y inversion issue, there is an option to invert the image in the DirectShow interface. Open NI-MAX, open the NI-IMAQdx tree and select cam1, then go to the Camera Attributes tab (bottom, center of the window below) and select All Attributes.

💐 cam1 : UI164xLE-C_4002842040 - Measurement 8	& Automation Explorer	A REAL PROPERTY AND		
File Edit View Tools Help				
Configuration	H Save H Revert I► Snap ►	🕨 Grab 🛛 🛴 Histogram	Save Image	📌 Hide Help
■ ● > System ■ ■ ■ </td <td>L Ord TX Diport Al 2] Sofresh</td> <td>Yew Cotons</td> <td>Title tage</td> <td>Ani - Mada Basics Mi - Mada Basics Mi - Mada Basics Mi - Mada Basics Sometarian comes Gondation (comes) Gondation (comes) Bet mu remote image policies</td>	L Ord TX Diport Al 2] Sofresh	Yew Cotons	Title tage	Ani - Mada Basics Mi - Mada Basics Mi - Mada Basics Mi - Mada Basics Sometarian comes Gondation (comes) Gondation (comes) Bet mu remote image policies
	Backlybt Compensation BrightCompensation Brightness Color Enable Contrast Denosure Gamma Gamma Sharpness White Balance	Al Attributes Camera Attributes Simple Intermediate Advanced Actual Names Display Names	۲ ۲	Expand All/Collapse All-Expands/collapses all of the autoclapsions in the tree control. The Explored bacteria and the second second second second Extension of the second second second second attributes while all of the attributes values. This battom can be useful for copating reads only attributes while an acquisition is in progress. Was Options-Configurate the view options of the attributes. All Attributes available for the correct second of the attributes. All Attributes available for the correct second of the attributes.

Then look for the 'Vertical Mirror' item in the 'Acquisition Attributes' entry and turn on the checkbox and then click 'Save' at the top center of the window.

Cam1:UI164xLE-C_4002842040 - Measurement &	Automation Explorer				_0×
Configuration	H Save H Revert I► Sn	ap 🕨 Grab 🛛 🛣 Histogra	am 🛛 🔓 Save Image		📌 Hide Help
Conjugation Conjugation Construction Con	Concernent of the second	h Wew Options	Hide Help Use hardware value 1024	x X X X X X X X X X X X X X X X X X X X) y roze neg
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Appendix - A/D Channel Setup

The A/D channels in the sensor sampling rules are offsets from the physical channels specified in the hardware setup. If you want to use different A/D channels then

- 1. Close ASET-LV4
- 2. Open ASET-LV4.ini (must be in the same folder as the ASET-LV4.exe file)
- 3. Change the channel settings in the sections described below
- 4. Save and close ASET-LV4.ini
- 5. Start ASET-LV4

ISP/PVP sensor types

Sensor 1 is controlled by the settings in the [Ion1 Setup] section, while Sensor 2 is controlled by the settings in the [Ion2 Setup] section. The section entries are as follows:

[Ion? Setup] D/A chan=0 A/D lo chan=1 A/D lo gain=10 A/D hi chan=0 A/D hi gain=100

The physical channels are used as follows:

Low gain from headstage	ISP AI Chan + A/D lo chan
High gain from difference amp	ISP AI Chan + A/D lo chan
Baseline signal	ISP AO Chan + D/A chan

D/A chan A	/D lo chan A/D	logain A/Dh 0 0	i chan A/D hi gair 100
Ion1 Ca	libration		
Slope -0.0112	Intercept 7 -0.64881	Efficiency 0.5	D cm^2-s 8E-6
Good Slo 58	ope Slope +/- 5		
wait sec 0.5	avg sec 0.5	base sec	cal sec
0.5	0.5	0.5	3

SVET sensor type

The SVET sensor type is only supported as Sensor 1 and cannot be combined with other sensor types at this time. Normally the section settings are as follows:

[SVET1 Setup] A/D start ch = 0 Cal sec = 3 Preamp Gain = 100 Main Amp Gain = 500

The physical channels are as follows:

X In Phase	SVET AI Chan + A/D start ch
X Quadrature	SVET AI Chan + A/D start ch + 1
Y In Phase	SVET AI Chan + A/D start ch + 2
Y Quadrature	SVET AI Chan + A/D start ch + 3
X Oscillator	SVET AI Chan + A/D start ch + 4
Y Oscillator	SVET AI Chan + A/D start ch + 5
Tip signal	SVET AI Chan + A/D tip ch

A/D start chA/D tip ch Cal Sec Preamp GainMain Amp Gain					
SVET1 Cal	ibration	,	,		
	Conductiv	/ity		Frequency	,
[5000	T	1	0	0
	uVolt per u	A	Phase Angle		
1	0	0	0	0	0
0	1	0	0	0	0
0	0	1	0	0	0
P		·	P	-	~

Optrode sensor type

Only one OptrodeExt sensor type can be used at a time, it cab be Sensor 1 or Sensor 2.

[DAQ] Optrode1 Sig Chan = 0 Optrode1 Ref Chan = 1

The physical channels are as follows:

Reference signal	Optrode AI Channels + Optrode1 Ref Chan
Measurement signal	Optrode AI Channels + Optrode1 Sig Chan
Drive signal	Optrode AO Channel