SVM340 Synchronized Video Microscope
User's Guide

Table of Contents

1. INTRODUCTION

The SVM340 is an inverted fluorescence microscope with built-in video camera, fluorescence filter, pulsed Light-Emitting Diode (LED) illuminator, motorized *x-y* traverse and focusing actuator. It can directly image fluorescent or non-fluorescent samples on a standard video monitor or video recorder.

In addition, the SVM340 includes an advanced programmable synchronization unit with four inputs and three outputs for synchronizing image acquisition to external events.

The basic functions can be controlled by the front panel controls or through the uScope application software included with the instrument.

1.1 New in uScope™Version 1.026

Version 1.026 of uScope™ software includes a number of new features and improvements to existing features:

Motion limits
Motion limits on the XY stage are useful if you have installed a device on the SVM (i.e. a chipholder) that may interfere with the stage at its preset limits.

Snap-shots
The snap-shot feature lets you create a single image consisting of an average of one or more consecutive images.

Time lapse
The Time lapse option lets you select the frame rate frequency to allow video taken over a long period of time to be compressed to a small file and played back at increased speed.

Video compression
Though uScope does not have built-in compression it can now use video compressors that are installed on your computer. To add compression

3

functionality, you need to download and install a "codec." A compatible codec will also need to be installed on anything that plays the video.

Deinterlacing
Improvements have been made to the deinterlacing method and to the Deinterlace Settings dialog box.

Buffering
Improvements have been made to the method by which video is buffered to RAM memory. The newest version of uScope software uses video buffering for the video recorded live (not from the pre-trigger buffer) so that this video is also saved without dropping frames.

Recording a video
Improvements have been made to the interface for recording pre- and post-trigger video.

Distance indicator
The Distance Indicator allows you to measure features on your image.

1.2   Package contents
The SVM340 package should include the following items:

- SVM340 microscope main body
- Camera module with one microscope objective according to your order
- Hauppauge WinTV PCI video input card and driver disk
- Power cable
- One RS232 serial cable and one S-video cable
- Installation disk with uScope software
- .This manual
- Extra objectives, illumination or camera modules as ordered.

If any parts are missing or damaged, please contact your local dealer or LabSmith immediately.

1.3 Basic functions

The SVM340 combines an inverted fluorescence video microscope with a programmable synchronizer and software for on-line image acquisition, processing and storage. You can use the instrument in several ways:

- As a stand-alone video microscope. Connect the video output from the SVM340 to an analog video monitor or VCR through the BNC or the S-video outputs on the rear panel. You can now focus, traverse and adjust illumination intensity by the controls on the front panel while observing the image on the monitor.
- As a software-controlled video microscope for automatic or manual acquisition of video sequences, using the on-line image processing and storage capabilities of the uScope application.
- As an integrated part of a complex experiment, synchronizing pulsed illumination, image acquisition and external devices in response to up to four trigger input signals.

In each of these modes, you can use microscope objectives with magnification from 4X to 20X, and acquire and store the video output on standard analog video storage hardware. With the uScope application, you can directly store the video data on computer disk as .aviI files and perform advanced real-time video processing.

1.4 Computer requirements

The SVM340 can be used with any computer equipped with a RS232 port or USB1.0 or greater port and a free PCI slot or USB2.0 or greater port for the video input card. However, since the uScope software is designed to stream digitized video sequences directly to disk, it is recommended that the computer fulfills the following minimum requirements:

- Windows XP, Vista or Windows 7 or newer
- One free PCI slot for the video capture card or a free USB2.0 port
- minimum of 1 GB of RAM (4-8 GB recommended)
- 80 GB hard disk with 12 ms access time (500-1000 GB recommended).

The SVM340 microscope includes a VGA-resolution analog CCD camera which outputs a standard RS170 monochrome or NTSC color composite video signal. The video signal is digitized by a DirectX9.0 compliant PCI video capture card or USB2.0 video capture peripheral, capable of digitizing and storing uncompressed VGA resolution video on disk.

Many PC video input devices include on-board image compression hardware, converting the video stream into various compressed video formats. Image compression standards like MPEG are designed for general visual imagery and may not be suitable for all types of imagery occurring in microfluidics device diagnostics, e.g., the images of isolated small particles as recorded in Particle Image Velocimetry (PIV) experiments. The ability to record uncompressed video is therefore an important feature of the hardware and software included with the SVM340.

Uncompressed video streams naturally take up more bandwidth and use more computer processing power for display and storage, so a powerful computer is recommended. When used on a newer standard PC with moderately fast CPU and disk speed, uncompressed video sequences can usually be stored on disk in real time. If used on slower computers, frames may be lost during recording. Slower computers may also exhibit a perceptible delay between an imaged event and its appearance on the computer display.

Also, a large hard disk is recommended for storage of video data. A color video signal will typically generate 1.6 GB per minute and thus quickly consume hard disk space.

1.5   Installing the software

1.5.1   Installing the video capture card and/or drivers

A video capture card or peripheral USB capture device is required to view and record the video signal on your computer. If you purchased one of the devices through LabSmith, a separate instruction manual with the installation instructions and driver disk will be included with the device. It

6

is always a good idea to check with the manufacturer's website to make sure you have the latest drivers.

If you have purchased a RS232-USB adapter you will also need to install the drivers for this device. Again, the disk with drivers and instruction manual will be included with the product.

Your computer may need to be restarted after the video capture or RS232 adapter drivers have been installed.

1.5.2   Installing uScope

If your computer does not already have DirectX9.0 or greater installed you will need to install it before running uScope. DirectX9.0 is free and available directly from Microsoft's website. Most computers will already have this software installed.

To install uScope, run setup.exe and follow the on-screen directions.

A dialog box will pop up that says "Select a new serial port". Select the COM port that the serial connector is connected to and click OK. If you are not sure which com port you are connected to … This dialog will keep popping up until you succeed in communicating with the SVM or you click Cancel (then you work offline and uScope does not try to send commands to the SVM).
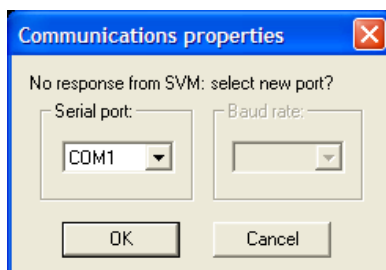
Communications settings dialog box

Note: Some functions are available with the SVM in offline mode. Manipulation of the stage (x, y, and z) and illuminator settings can

be accomplished via the front panel. Images can be viewed and recorded with the SVM operating offline, however real-time measurements (PIV and Intensity probes) are disabled.

2   SVM340 HARDWARE

The following section describes how to set up your SVM and how to use it independent of the uScope software.

2.1   Feet

The SVM340 is shipped with the feet removed to protect the unit during shipping. They must be installed on the unit before an optics module is inserted.

Note: The SVM uses a sheet metal chassis to minimize weight and cost. The sheet metal chassis occasionally can have some unevenness that causes it to rock when the feet are attached. The feet are designed to have some adjustability to accommodate for this unevenness. This unevenness does not affect the function of the SVM due to the kinematic design of the focus stage.

2.2   Illuminator module

The illuminator module consists of four independent LED channels. Two of the channels (A and B) have 8 LEDs each and two channels (C and D) have 4 LEDs each.

Available LED colors include:

Color	Wavelength range
Blue	464-476 nm
Green	520-535 nm
Red	615-636 nm
Yellow	
White	

Standard illuminator modules:

Name	Channel A	Channel B	Channel C	Channel D
LED-B	blue	blue	white	Blue
LED-G	Green	Green	White	Green
LED-R	Red	Red	White	Red
LED-Y	Yellow	Yellow	White	Yellow
LED-W	White	White	White	White
LED-X	Blue	Green	White	red

LabSmith can provide customized illuminator modules with any combination of LED channels.

The illuminator module can be removed from the traverse from the top by pulling the module upwards.
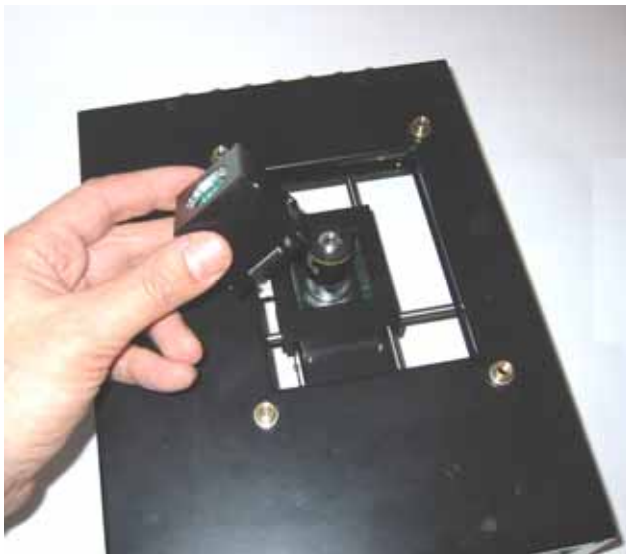
Removing the illumination module

Note: When reinserting the illuminator module take care that the connector pins are all correctly inserted in the receptacle without bending or damaging the pins. Also take care not to apply excessive force. Support the camera module from below with your

hand when inserting the illumination module and press from below to ensure the traverse mechanism is magnetically seated.

2.3 Optics module

The optics module is attached to the traverse by magnetic holders and can be removed by tilting the SVM340 on its side and gently pulling the camera module down from below the SVM340 body until it comes free.

Important! Before removing or inserting a optics module turn off the SVM340 using the power button at the left of the front panel.

Optics module

Note: When removing or inserting the optics module, take care not to apply excessive force since this may damage the traverse mechanism and compromise traverse accuracy.

2.4 Microscope objective

The microscope objective is a standard DIN type objective with 160 mm conjugate image distance. To replace the objective, remove the camera module and unscrew the objective. The SVM340 supports objectives with magnifications from 4× to 20×. Objectives with higher magnification generally have insufficient stand-off distance to clear the illuminator LED's and can only be used with an external illuminator module or other external light source.

WARNING: Dust particles can enter the optics module when the objective is not installed. Removal or changing of the objective should be done in a clean environment when possible. We recommend that a piece of tape be placed over the objective opening if the optics module will be stored without the objective installed.

Objective and seat for fluorescence filter

2.5 Fluorescence filter

The fluorescence filter can be installed in the B&W and Color camera modules. The filter is installed by removing the objective and carefully

placing the filter in the recessed opening. A compliant washer (provided with the filter) is placed over the filter to keep it secure under the objective.

Note: The EPI camera module is supplied with pre-installed fixed filters that cannot be removed.

2.6   Base stand

Power: plug the power cable from the back of the SVM340 into a 90 – 240 VAC power outlet.

Communications: connect the 9-pin D-sub serial connector on the back of the SVM340 to a free serial port on the PC. You may use a USB/RS232 converter device if your computer does not have a RS232 port.

Video: the connect the s-video cable to the S-video input on the back of the SVM and into either the s-video port on the video capture card installed in your computer or the USB video capture device. For the later, then plug the USB video capture device into a USB port.

Most of the access to the SVM340 features will go through the uScope application software. You can also, however, access some important functions directly from the front panel.

SVM340 front panel

2.7   Front panel controls

The Power button and Power LED are located in the upper left corner of the front panel. When the power is turned on, the LED will flash green and red while the system runs its initial tests, and turn green when the tests have passed.

Site-Light   These buttons toggle between SITE mode and LIGHT mode. When the SITE button is lit, the four storage buttons A–D represent four different stored positions, and the keypad controls the traverse movements. The focus buttons moves the objective up and down for focusing.

When the LIGHT button is lit, buttons A–D represent the four LED channels, and the keypad up and down buttons control the intensity of the LED banks selected by the A–D buttons.

A–D   In SITE mode, the buttons A–D represent four stored

13

settings of the traverse and focus positions and LED intensities.

To recall a stored position, select SITE mode and press and release a storage button. The traverse will move to the location and set the four LED intensities to the values of the stored settings.

To store a traverse position, press one of the storage buttons and hold it down a few seconds until the button light goes off. This will store the current traverse position and the LED settings in the selected storage cell.

Note: There are a total of 10 preset positions available on via the uScope software.

In LIGHT mode, the buttons A–D represent four LED channels, labeled A-D. The 24 LEDs in the illuminator module are divided into four channels, which can be controlled individually. When a LED bank is selected, the corresponding button lights up. One or more LED banks can be selected simultaneously by pressing the one or more of the A–D buttons.

Note: When an EPI Optics Module is installed in the SVM, the EPI illumination is controlled using front panel button D. In this case illuminator channels B and D are both controlled by front panel button B.

Keypad   In SITE mode, the four buttons will move the traverse in the x and y directions. Pressing a button will start the traverse motor at low speed, and after about two seconds motor speed slowly ramp up to high speed. Pressing the button briefly allows single stepping of the traverse.

Pressing the center button (Stop) will immediately stop any traverse movement which may be in progress.

When in LIGHT mode, the up/down keys will increase/decrease the intensity of those LED banks which are selected by the A–D buttons. One or more of the LED banks can be controlled simultaneously.

Pressing the center button (Stop) with switch off all selected LED banks.

Focus up/down   These buttons will move the focus motor up or down to focus the image. The focus motor will start at slow speed and then ramp up to high speed.

Lock indicator   The lock indicator LED on the front panel is green when the SVM340 is in position.

The Lock indicator LED turns red when the SVM340 is in motion, e.g. while the traverse is moving to a preset.

2.8   Back panel connections
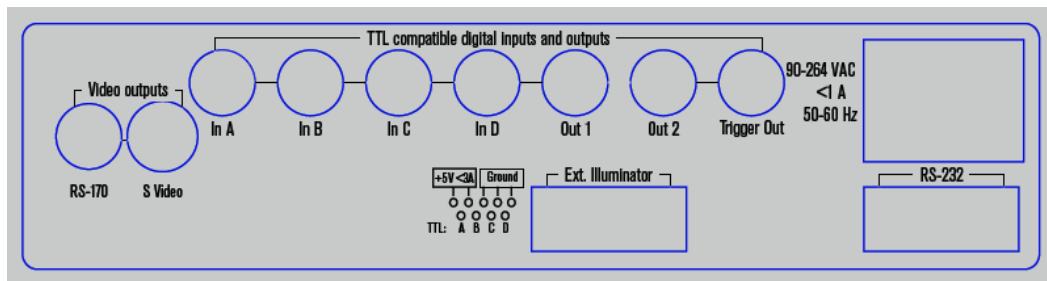
The SVM340 back panel

15

2.8.1 Video output

The video signal is output on two connectors: a BNC connector with composite, analog RS170 or NTSC video, and an S-video output compatible with most video cards and analog recorders. It is generally recommended to use the S-video for best image quality, but some monitors without S-video input may require the composite video signal.

2.8.2 Digital inputs and outputs

The four digital inputs and three outputs on BNC connectors provide TTL level communication with external equipment. The inputs can be used to control or strobe the four LED illuminator channels or to trigger more advanced behaviors. The outputs are selectable and include video timing information, motion status information, and several advanced programmable flags. The inputs could be connected to digital experiment controllers like the LabSmith LC880, interlock switches, sensors, or other external devices, and the outputs connected to other apparatus to facilitate real-time control and automation.

2.8.3 External Illuminator

This male nine-pin D-sub connector provides 5 V DC power and pulse signals to drive 4 external illumination sources with settings similar to the four-bank led module.

Pin connections

1	5 V DC (max 3 A)
2	Chassis Ground (0 V)
3	Chassis Ground
4	Chassis Ground
5	Chassis Ground
6	LED A drive (TTL)
7	LED B drive (TTL)
8	LED C drive (TTL)
9	LED D drive (TTL)

16

The light intensity of the SVM340 LEDs is controlled by pulse width modulation with a frequency synchronized to the video signal. Full light intensity means an illumination duty cycle close to 100%.
LED drive outputs A–D are negative logic, i.e., TTL level is high when LEDs are off.

2.8.4   RS232 serial connector

The female 9-pin D-sub connector is for RS232 communications. This link allows the SVM340 to receive programming and commands from an external controller, e.g., a computer running the uScope application or LabView through the provided serial cable. A USB/RS232 converter can be used for computers without an RS232 connector (COM port).

2.9   Microscope stage

The SVM340 is fitted with a replaceable microscope stage, attached to the main body of the instrument by four magnetic locks. To remove the stage, simply pull the stage gently up until it releases. The stage top is a polished stainless steel plate, which can be machined to provide application-specific mounts for the fluidic device, electrodes, fluid hoses or other fittings.

Removing the microscope stage

7.00" × 9.00" rectangle to place the instrument on a plane table surface. The feet can be replaced by leveling feet which allow fine adjustment of the instrument or standard optical posts which can be clamped firmly to an optical table. Contact LabSmith for more information.

2.10 Motion limits

To restrict the motion limits on the XY stage select SVM>Motion Limits… Modification of these limits may be useful if you have installed a device on the SVM (i.e. a chipholder) that may interfere with the stage at its preset limits.

3   VIDEO AND ILLUMINATION TIMING

The master clock for the SVM340 is provided by the video signal timing. The CCD camera outputs video in standard RS170 format (NTSC in the color version), which is an analog, interlaced format compatible with standard analog video monitors or video recorders.

18
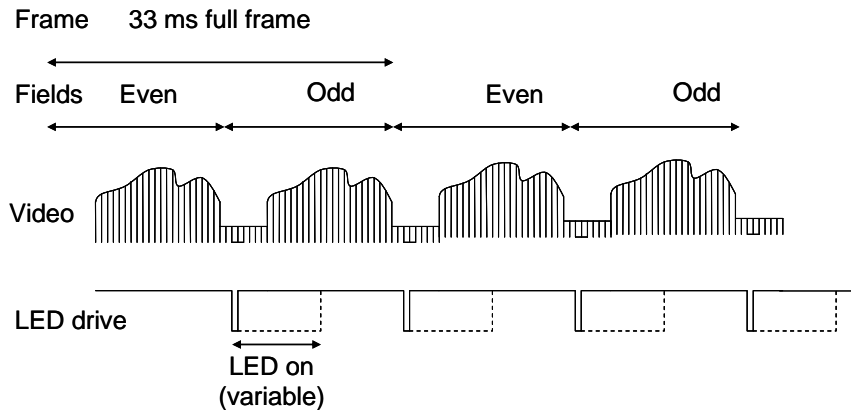
Timing sequence of the illumination in relation to the video signal

The RS170 interlaced video signal is composed of two fields, called even and odd fields, each containing every second line of the image. The interlaced format was defined in the early days of television to avoid flickering TV images. The even field contains lines 0, 2, 4, …524 and the odd field lines 1, 2, …525. The field frequency is 60 Hz, with one even and one odd field adding up to a full video frame each 33.3 ms, corresponding to 30 Hz frame frequency.

To ensure that all lines of the video signal are equally illuminated, the LED's flash twice during an image, once in every field. The LED pulse starts in the frame blanking period, and its width can be varied from 0 to near 100% of a field period, 16.6 ms.

Due to the interlaced readout of the camera sensor, images of fast moving objects, which move a noticeable distance during the 16.6 ms between two consecutive fields, may appear jagged at the horizontal edges. See section below for a discussion of the implications and tools to control the interlacing effect.

4   uSCOPE SOFTWARE

The uScope software lets you set the functions of the SV340 and control the video acquisition and on-line processing. It also allows you to recall

and process stored video files. uScope runs on any PC with Microsoft Windows XP operating system or later.

> *Note:*   The uScope application makes extensive use of the DirectX software, which is provided by Microsoft Corp. and installed independently of uScope. If you have installed a local language version of Windows XP, DirectX will install in the same language. Consequently, some of the dialog boxes shown below may appear in the language of your windows installation.

The uScope main window is divided into the following sections:

- The video display window
- The LED control panel
- The focus control panel
- The presets panel
- The video recording panel
- Probe controls.
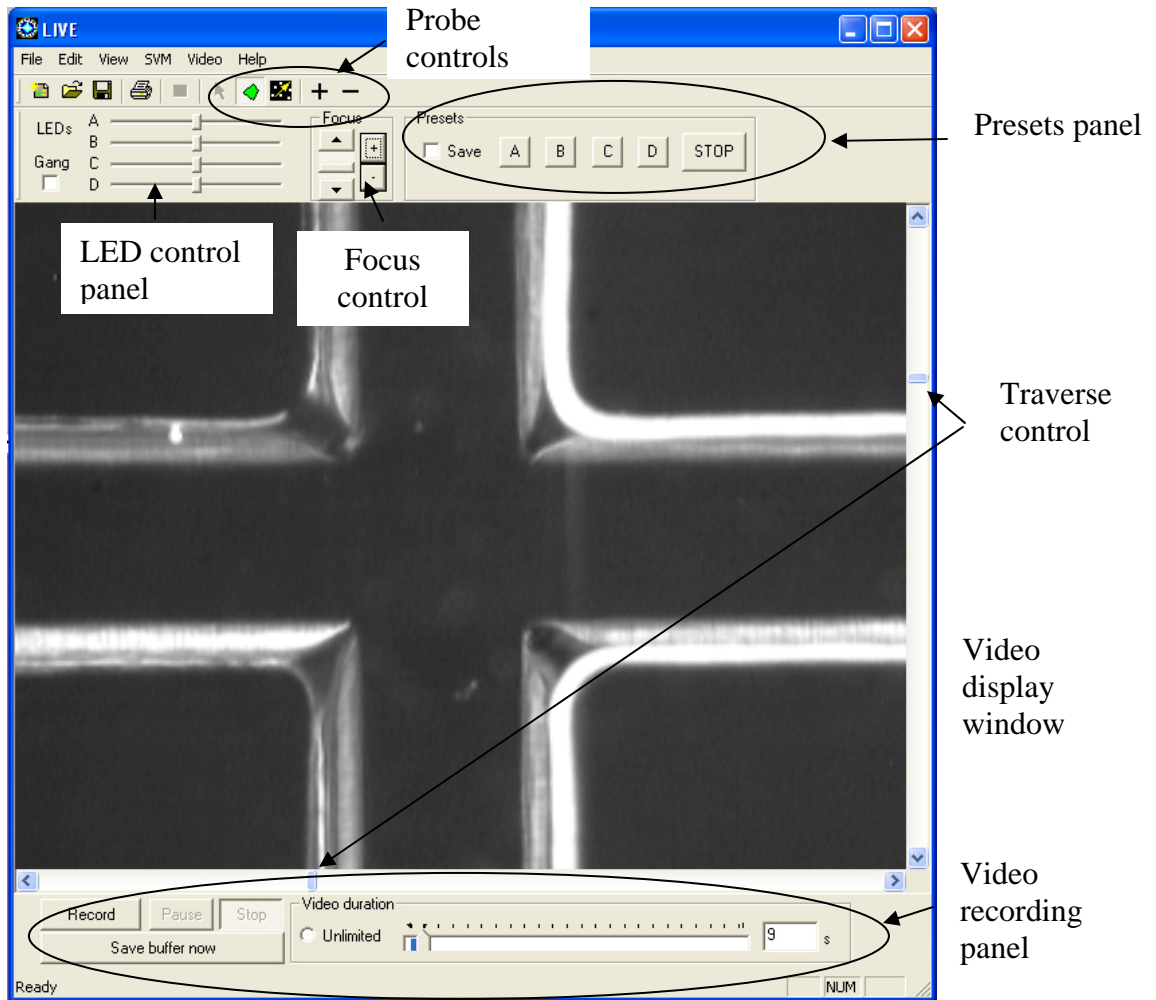
uScope main window

The video display window shows the off-line or on-line (live) video as
selected in the Video menu.

X-Y Traverse. The position indicators to the right and below the video
display indicate the position of the *x-y* traverse and can be used to move
the traverse.

The traverse can also be adjusted using the keyboard arrow keys. Holding down the shift-key while using the arrow keys slows the traverse to allow fine adjustment.

The X-Y traverse has a motion range of 50 mm by 75 mm. This range can be limited by selecting SVM>Motion Limits and adjusting the slider bars. The Motion Limits dialog box is shown below.
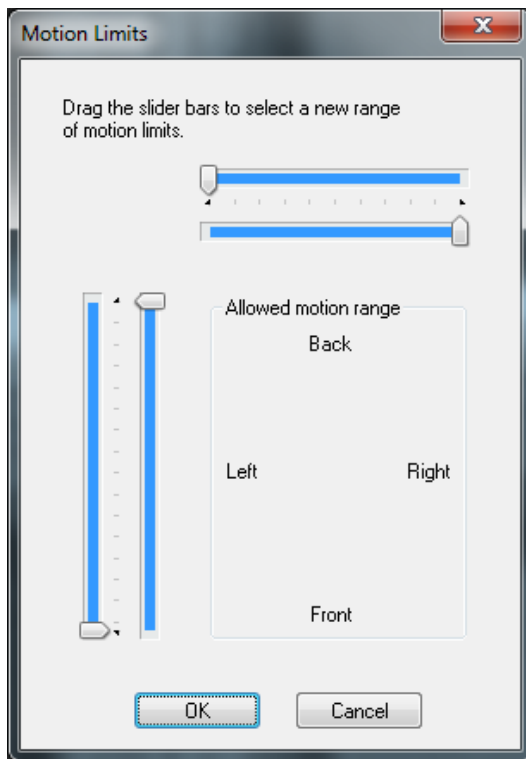

Motion Limits dialog box

Focus. The focus indicator slider bar is located at the top of the main window. The slider bar can be used to adjust the focus, while the + and – buttons to the left of the bar are used for fine adjustment.

22

The focus can also be adjusted using the Page Up and Page Down keys on the keyboard.

Illuminator. As described in the Video and Illumination Timing Section above, LED illuminator channels strobe during each image frame. The Illuminator control is used to adjust the strobe duration of each channel, effectively altering the LED intensity.

When a standard B&W or Color optics module is installed, the control panel slider bars at the top are used to adjust each LED channel, A–D as shown below.
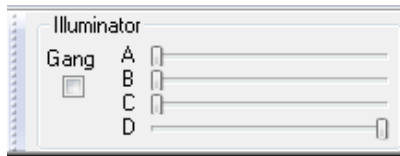
Checking the Gang box will cause all LED banks to be adjusted simultaneously when one slider is activated.

When an EPI optics module is installed, the illuminator control will be as shown below.
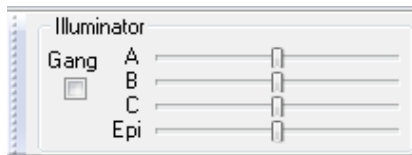
For this case the channel "B" slider bar adjusts both the B and D channel LEDs.

Note: The Illuminator scroll bars do not update when the illumination is changed via the SVM front panel. If the front panel will be used for illuminator control, we recommend first setting all illuminator slider bars to the maximum setting to allow the full range of illuminator control.

The illumination can also be adjusted to a specific level using the menu SVM>LED Settings. The following dialog box will appear:
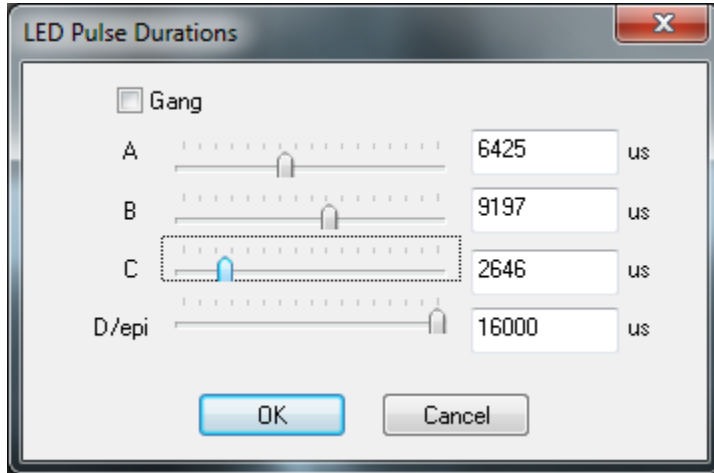
LED Pulse Durations dialog box

The maximum illumination setting is 16600 us.

Presets. The presets panel allow saving and restoring up to ten traverse (x, y, and focus) positions and illumination settings in storage cells A–J. Clicking a button will load the stored preset and adjust traverse position and LED illumination to the stored values. Checking the Save box first will store the current setting in the selected storage cell. Sites A-D can also be saved and accessed from the SVM front panel.
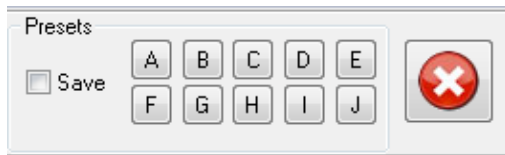
Click the Stop Motion button to stop the traverse.

For a description of the Video recording and probe functions, see sections 6 and 7 below.

4.1   usc files

The uScope software saves the instrument and video settings in a file with .usc extension. To open a uScope file, choose File>Open, then locate the file on your hard drive. You can also choose from recently opened files at the bottom of the File menu.

4.2   Online and offline operation

uScope can work in both online and off-line mode. When on-line, it communicates with a SVM340, controls its functions and accepts live video signals from a DirectX compliant video capture card.

When off-line, uScope can open a stored video file for playback and further processing. uScope will go into off-line mode whenever it fails to locate a SVM340 on the selected serial port.

4.3   Upgrading firmware

The firmware is the software stored inside the SVM340 in non-volatile memory and controls the internal functions of the instrument, such as traverse movements, front panel lamps and buttons, and back-panels inputs and outputs. The firmware is included in the uScope application package and can be loaded into the SVM340 from within the uScope software.

To upgrade firmware to the latest version:
1. Download the newest version of uScope from www.labsmith.com and install on your computer following the installation procedure described in the introduction.
2. Connect and turn on the SVM340 and start up the uScope application on the PC.
3. In online mode, choose SVM>Update Firmware>Update All
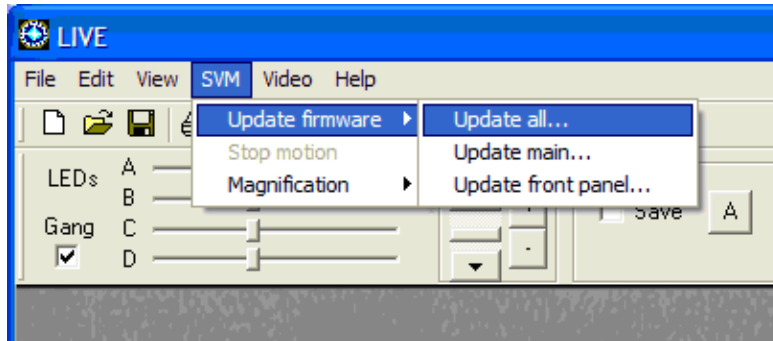
4. Click OK when the update dialog box appears
5. Wait while the firmware is updated. You can follow the progress in the status bar at the bottom of the uScope main window.
6. When the progress indicator reaches 100%, the upgrade is completed.

Important: Do not turn off or disconnect the SVM340 or the PC while the upgrade is in progress. This may result in loss of communication with the instrument that requires LabSmith assistance to resolve.

5   RUNNING uSCOPE SOFTWARE

When you run the uScope application, it will automatically connect to the SVM340 if it is present on a serial port and turned on. If uScope does not find a SVM340, the communications settings box will appear as shown above. Select the correct serial port and press OK.
The uScope main window below will appear.
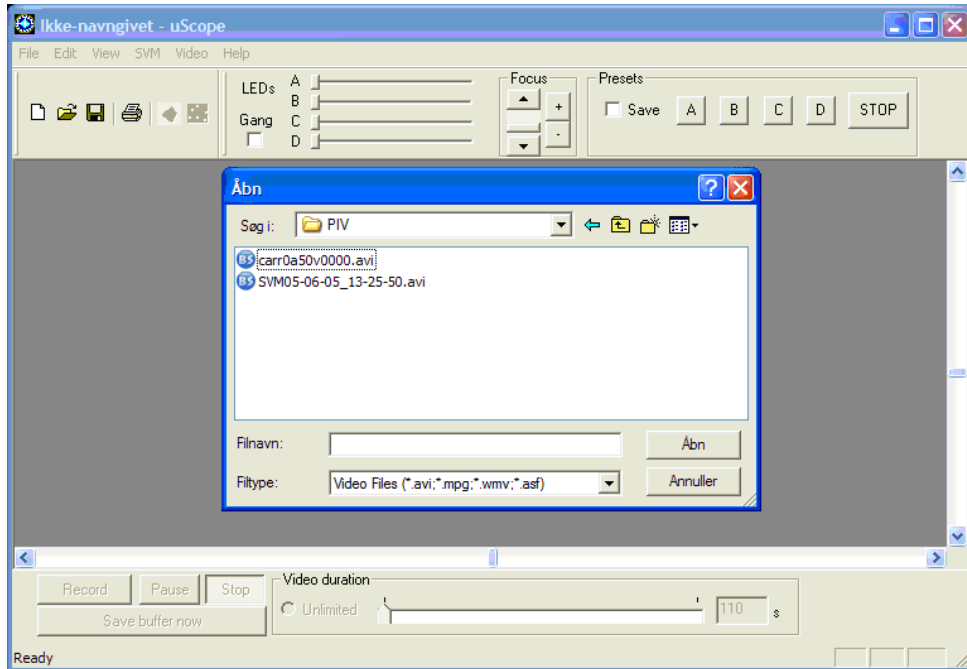
For off-line mode, open the AVI file you want to process off-line. The SVM340 does not have to be connected.
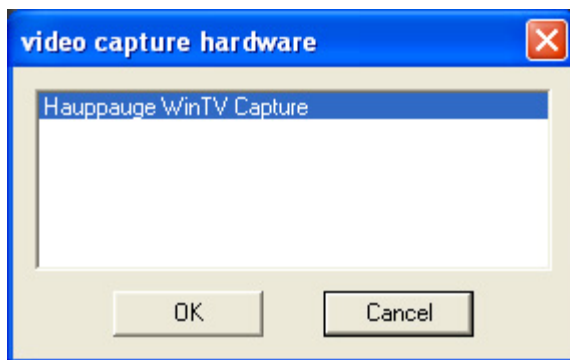
For on-line (LIVE) mode, click cancel in the "Open" dialog box for on-line operation, and click Video>Process live video.

5.1   Video options set-up

If the "video capture hardware" dialog doesn't pop up (it should the first time) click View>Video options>Input device.
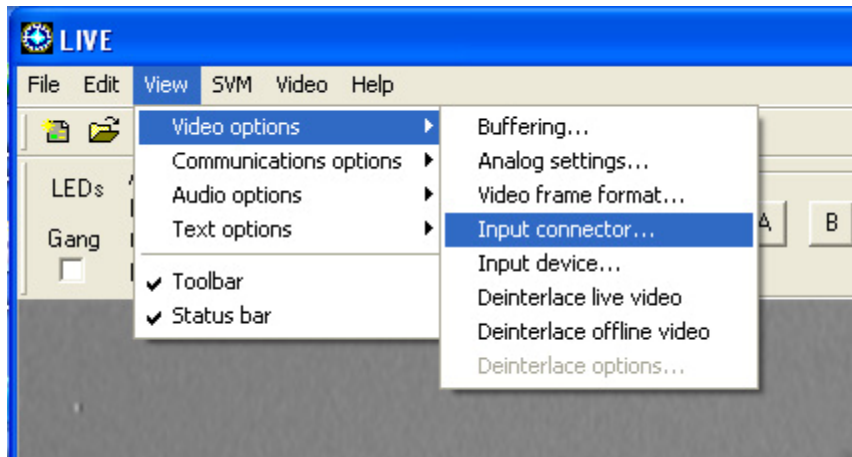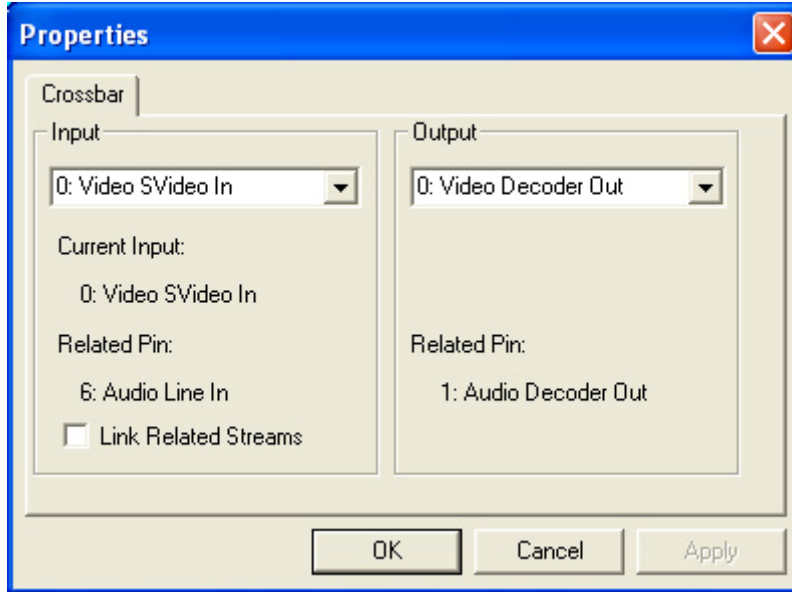
Video options menu

Click on the video capture card that you want to use. Select the appropriate capture source.

Make sure that either the BNC composite or S-Video connector output of the SVM340 is connected to an input of the Hauppauge card, and select the relevant input connector through the Input connector box. Click View>Video options>Input connector and select the input that you want (e.g. Input: 0: Video SVideo In, Output: 0: Video Decoder Out). Click OK. At this point you should see live video on your screen.

Input connector selection box

You do not have to worry about the audio options since they are not currently used for the uScope application.

To set the video frame size you can click Video>Video options>Video frame format. Select 320×240 for low-resolution images and 640×480 for full-resolution images. Do not change the color space (whatever comes up, normally RGB24, is correct).

Note:	The DirectX video controls dialog boxes are of general nature and allow settings incompatible with the SVM340. Do not change the Video Standard (NTSC_M) or the output aspect ratio (the size must be 640×480, 320×240, 160×120 or 80×60)

5.2   Color format set-up

The SVM340 can be fitted with either a grayscale (B&W) or color camera, and the video output should be displayed and stored in corresponding monochrome or color formats. This is done by means of Look Up Tables

(LUT's), which convert the analog voltage outputs of the video signal to the appropriate gray scale or color values.

If you have a B&W camera, you want to make sure you are using an 8-bit LUT, converting the analog voltage into 256 different gray scale levels. Normally you would use a grayscale LUT, mapping the 256 gray scale levels into 256 different shades of gray, but you can apply color LUT's for false color display. The reason for using false color display is that it is difficult to distinguish 256 different gray levels on a standard computer monitor. Converting shades of gray into colors can significantly enhance visibility of small differences in gray scale value.
You can experiment with false color display by: Click Video>Color format>Spectrum LUT, etc.

If you have a color camera and you want to keep the color, click Video>Color format>24-bit RGB. You may also select a monochrome 8 bit with a color camera to save RAM and disk space. Color video data takes up 8 bits each for the red, blue and green colors, and thus consumes three times as much disk space and processing time as 8 bit-per-pixel video.

6   VIDEO RECORDING

One of the main features of the uScope application is the ability to record long, unbroken video sequences without compression. These video sequences are stored in standard AVI format, so that they can later be viewed by Windows Media Player or other video playback software, off-line processed by uScope or other video processing software, compatible with the AVI standard.

To enable uncompressed video recording, uScope makes use of a double buffering system, described below. The buffering scheme also enables pre-trigger recording, enabling you to store a video of what happened before the trigger instant.

6.1   Video compression

uScope can use video compressors that are installed on your computer, but does not have any built-in compression. To add compression functionality, you need to download and install a "codec." A compatible codec will also need to be installed on anything that plays the video.

All codecs are not equal. Some compress well. Some compress fast. Avoid over-compressing the data. The compression occurs when the video is being captured, so it cannot be "undone" if the results are not desirable. If you plan to perform PIV on your data we recommend using a lossless compression. The best-quality video compression takes time. It is often best to save videos with minimal or lossless compression and later post-process them.

Video compression codecs we recommend include:

Xvid (www.xvid.org). Excellent open-source compressor.

Lagarith (http://lags.leetcode.net/codec.html). High-performance lossless compressor.

Virtual dub (www.virtualdub.org). Excellent video-post processor and editor.

To save a video with compression, select Video>Use Video Compression… and the following dialog box will pop up.
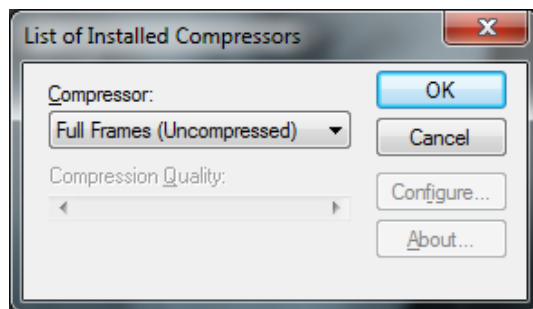
List of Installed Compressors dialog box

Select the desired compressor from the drop down box. The options listed in the configure dialog box will depend on the installed compressor. Questions regarding specific compressor settings should be directed to the compressor distributor.

6.2 Recording speed

A standard monochrome RS-170 video signal converts into a digital data rate of 8.9 MBytes/s, which can easily be read into PC RAM memory in real time. It is also within the capability of modern, fast computers to write to hard disk in real time at this data rate, provided the computer is not overloaded by simultaneously executing other disk or CPU intensive tasks. Color NTSC video signals convert into a data rate of 26.4 MBytes/s, which can readily be written to RAM memory in real time, but may be too high to write to hard disk in real time. In that case, some frames scattered throughout the video sequence are lost, resulting in a stored video sequence with time intervals of 33.3 ms between most images, but with 66.6 ms, 99.9 ms or some other multiple of the base frame interval between some individual images.

Such lost frames are called dropped frames. Video sequences with dropped frames are not suitable for accurate time history analysis, since it is difficult to know afterwards exactly where frames are missing.

6.3 Buffering

The SVM's uScope software uses buffering to capture images without dropping frames. uScope stores digitized video date in a cyclic RAM buffer simultaneous with the display. Buffering also allows the user to selectively record events after they occur. For instance, to catch a particular particle or droplet passing through the field of view, the user can set uScope to start recording from a number of seconds before the record button is pressed and then wait to start recording until the event has been observed.

The duration of the buffered video sequence depends on the amount RAM set aside for the buffer. The more RAM installed in the computer, the

more buffer space can be set aside without slowing down other tasks. The initial buffer size is set to half the available RAM, but you may want to adjust the buffer size get longer pre-trigger video sequence duration. Buffer size is specified in The View>Video Buffering settings menu.
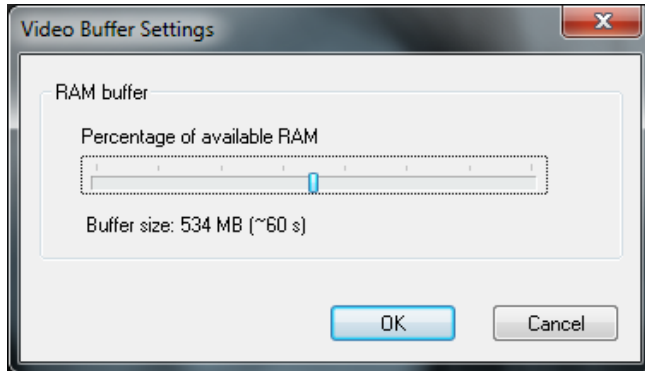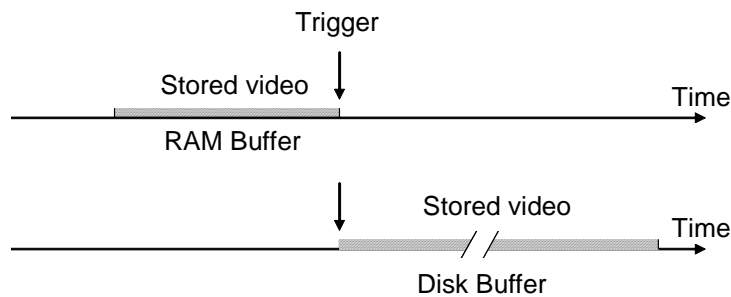

Video Buffer Settings dialog box

Note: Choosing the 24-bit option for recording video (Video>Color Format>24-bit RGB (true color) requires 3-times the buffer RAM of all other video options.

6.4   Pre- and post trigger recording

The figure below illustrates the effect of the buffer size on the pre- and post trigger recording durations.


Pre-trigger (top) and post-trigger (bottom) recording

For pre-trigger recording, you save the video sequence occurring before the trigger, stored in the RAM buffer. With post-trigger recording you save the video sequence occurring immediately after the trigger and temporarily stored in the disk buffer. The disk buffer is normally larger than the RAM buffer.

6.5   Recording a video

To record a video, set the time in the *Record from* box to the desired number of seconds, or drag the slider bar between now and the maximum buffer size. This will set the time duration that is recorded from the pre-trigger buffer.
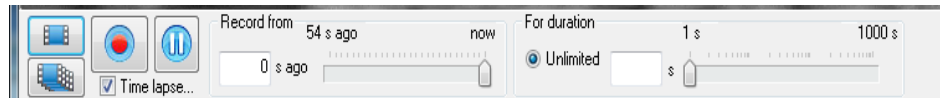
Then either set the *For Duration* time, or leave it as Unlimited. The duration time represent the total video duration (pre-trigger buffer + post trigger record time). If Unlimited video duration is used, the video will record until the record button is pressed again. The total duration of the video will be the buffer time plus the newly recorded time (the time the video button is depressed).

*Note***:**   uScope software now uses vido buffering for the video recorded live (not from the pre-trigger buffer) so that this video is also saved without dropping frames.

6.6 Snap-shots

A snap-shot is a single image consisting of an average of one or more consecutive images.

Snap-shots are taking by pressing the [image] or [image] button, both located in the lower left hand corner of the main window.

[image] Takes a snap-shot using the predetermined frame averaging and other options specified in the Snap-Shot Settings (see below)

[image] Takes a snap-shot and frame averages until the button is pressed again. All other options specified in the Snap-Shot Settings (other than Number of frames to average) are maintained.

Snap-Shot Settings. The dialog box is opened from View> Snap-Shot Settings.
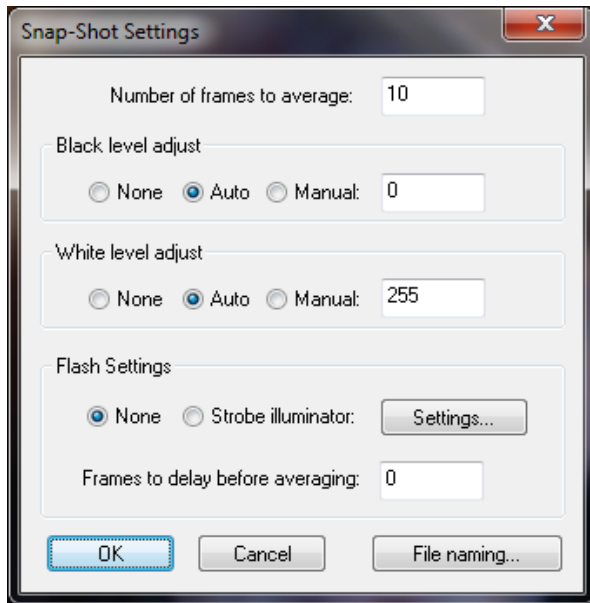
Snap-Shot Settings dialog box

Number of frames to average. This is the number of consecutive frames averaged to form a single snap-shot. If you have motion and are trying to capture particle images this number is typically 1 (no averaging). However, if you have a little to no motion, increasing the number of frames will improve your signal to noise ratio (for static images the signal to noise ratio will improve by approximately the square root of the number of frames averaged).

Black and White level adjust. Typically not changed. Use these values to change limits of the video spectrum. In the full color spectrum 0 represents black and 255 represents white. If this range is reduced everything below the set black level will appear black and everything above the set white level will appear white.

Flash Settings

   None: Illuminator will not change when snapshot is taken.

   Strobe illuminator: Illuminator settings will change when snapshot is taken. Selecting Settings button opens the LED Pulse Durations dialog box, where the snap-shot illuminator settings can be selected. After the

snap-shot is complete the illuminator will switch back to the original settings.
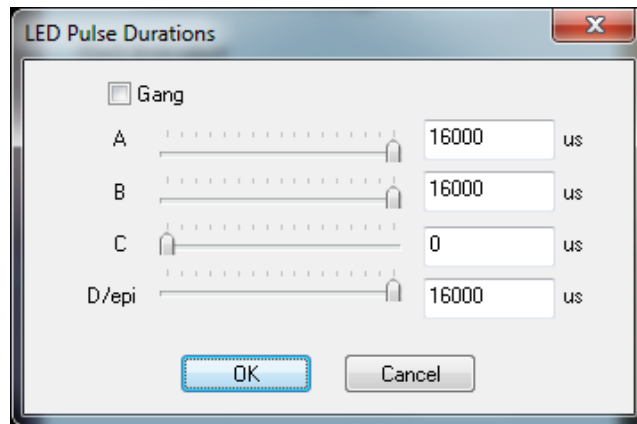

LED pulse duration dialog box

This function is useful to prevent photobleaching or heating of your sample. With this option the illuminator is typically turned off or very low prior the snap-shot.

Frames to delay before averaging. Choose the number of frames to skip after the snap-shot button is selected and before it takes the photo.

File naming. This option allows you to set the default for the type of file, compressed (jpg) or uncompressed (bmp) and the naming. The files can be autonamed or you can choose to be prompted each time to choose the file name. This window can also be access from File>Snap Shot File Saving…
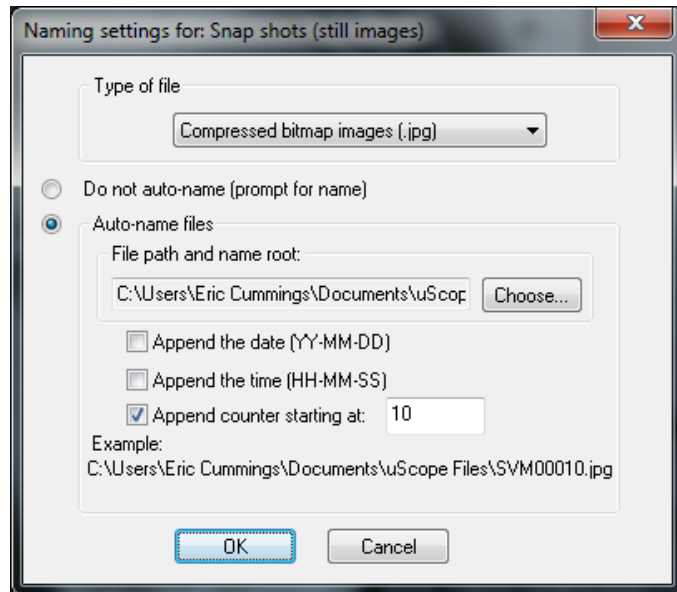
Naming settings for snap-shots

6.7 Deinterlacing

The camera built into the SVM340 runs in standard RS-170
(monochrome) or NTSC (color) video format at a fundamental frame rate
of 30 Hz. As discussed in section 5.1, the full video frame is composed of
two interlaced fields at a field frequency of 60 Hz. This means that every
second line of a full image is recorded at a time 16.6 ms later than the
other half of the lines. As a consequence, fast-moving objects will be
recorded with a slight horizontal blur, which is caused by the image
segment in the even lines being shifted slightly from the image segment in
the odd lines.

38
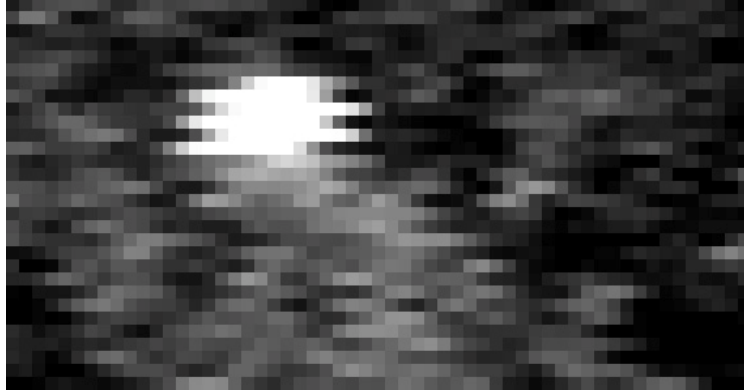
Image of a horizontally moving particle recorded with the SVM340 interlace camera

Zooming in on such a fast-moving particle reveals the jagged edges caused by the interlace camera format.

To reduce the effects of the interlacing, uScope includes deinterlace filters, which will reduce the visual appearance of the blurring caused by the interlacing by various algorithms. The delinterlace method is selected in the Video>Deinterlace Video options dialog box.
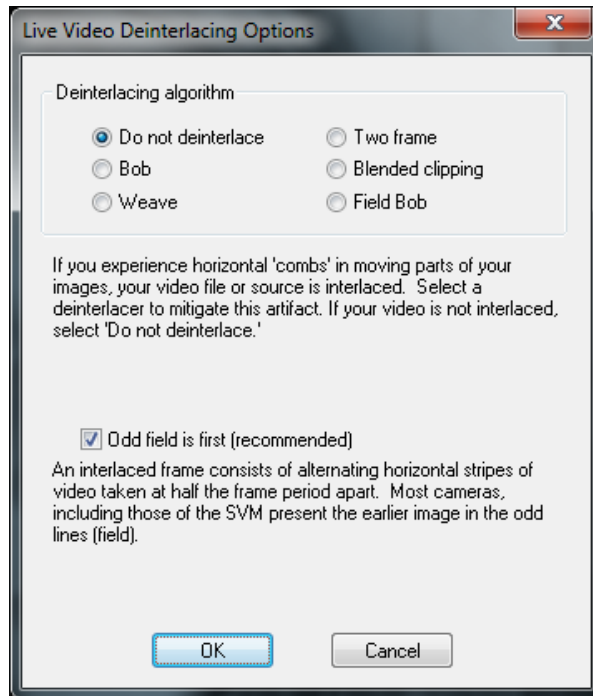
Live Video Deinterlace Video options dialog box

The best deinterlace filter depends on the nature of the video image and should be chosen by experimentation. A brief description of the three most common algorithms is given here.

Weave. This method uses three fields in the calculation and works well on slow moving material but tends to fail on fast moving material.

Bob. The basic bob algorithm uses the most recent field and fills in the lines between by interpolation. This method detects weaving artifacts in the current image it uses bob to get rid of them. This method has a tendency to bob rather too much and gives poor results on fine static images.

Two-frame. This method uses the current frame and the last two to determine whether to bob or weave a given pixel. This gives better results

40

on both stationary and moving images than the above two methods but uses more CPU.

Although the deinterlace filters improve the visual appearance of the video, they are not always effective for scientific imagery. All filters are based on some form of interpolation between frames under the assumption that scene motion is continuous between frames. When the movies are analyzed by various algorithms, the effect of the deinterlace filter on the result will be algorithm-dependent.

6.8   Time lapse

The Time lapse option is used to select the frame rate frequency to allow videos taken over a long period of time to be compressed to a small file and played back at increased speed. The time lapse options are set by clicking on the Time lapse… button(☑ Time lapse…) at the bottom left side of the main window. The Time Lapse Settings dialog box will appear.
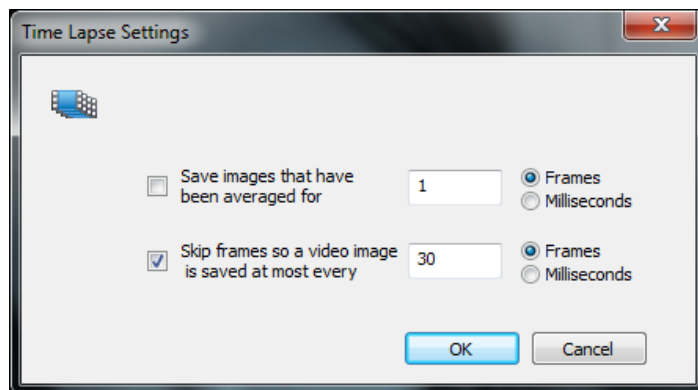
Time Lapse Settings dialog box

If averaging is desired, check the top option and select the number of frames or miliseconds to average. The resulting file will have an apparent increase in speed equal to the number of frames averaged images.

If frames are to be skipped, check the bottom option and select the number of frames or milliseconds to skip. A setting of 1 indicates one frame is

skipped between each captures frame. The resulting file will have an apparent increase in speed equal to the number of frames skipped between images +1.

If both options are checked, the set number of frames will be averaged, then the set number of frames will be skipped, resulting in an apparent increase in speed of the frames averaged + the frames skipped.

6.9   Distance indicator

The Distance Indicator feature allows you to measure features on your image. To create an indicator:

1. Click the **Distance Indicator** toolbar button ⬚ to highlight the button.
2. With your mouse curser over the image, right click on the mouse and select New Probe. Click on the image to insert the indicator. The ends of the indicator can then be moved (click and drag) to the desired locations.
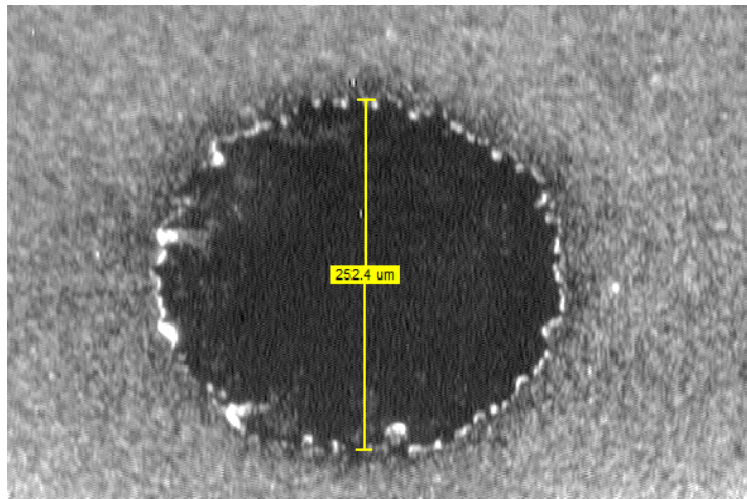
New distance indicator probe

> *Note*:   The distance calculation is based on the magnification setting for the objective. If the magnification setting is incorrect the

distance will be incorrect. If you are evaluating a saved image you will need to know what magnification was used and adjust the magnifiction at SVM>Magnification>…

3. Repeat step 2 to create as many indicators as desired.

Distance Indicator display data can be selected by selecting the probe (left click), then right click and select Properties. The following dialog box will appear.
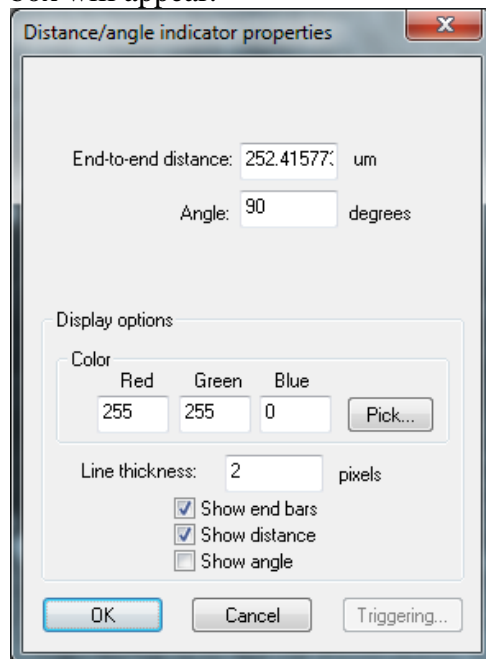
Distance/angle Indicator Properties dialog box

7 PROBES

uScope has sophisticated real-time probe capabilities that allow users to monitor image properties like color, intensity, variation and video properties like inferred motion (e.g., particle image velocimetry) in real time. These real-time measurements can be recorded to disk and can

43

trigger real-time actions. uScope can support as many probes as your computer's processor can handle.

The two types of probes that can be made in uScope are the Velocity (PIV) probes and Intensity probes.

7.1   Velocity (PIV) probes

uScope software makes it easy to create probes to monitor flow characteristics. Each probe can have its own properties; probes are almost always square, though it is possible to extend the probe in one direction to increase signal-to-noise ratio along that axis.

TIP: When learning how to use uScope's PIV probes it is useful to start with a stable particle flow, or a movie of a stable flow.

7.1.1   Setup

1. Connect the SVM to the computer and turn on both.
2. Launch uScope software.
3. Prepare the microfluidic channel. Fill it out with the buffer and introduce a sample of polystyrene fluorescent particles.
4. Adjust focus, illumination and flow characteristics.

Alternately, you can also open an existing flow movie by choosing **Video > Process Saved Video** Creating velocity probes.

7.1.2   Creating a PIV probe

To create a probe:

1. Click the **Velocity Probe** toolbar button to highlight the button.
2. With your mouse curser over the image, right click on the mouse and select New Probe. Bring the mouse to the center point for the new probe and left click the mouse to place the probe. A probe such as the one below will appear.

44
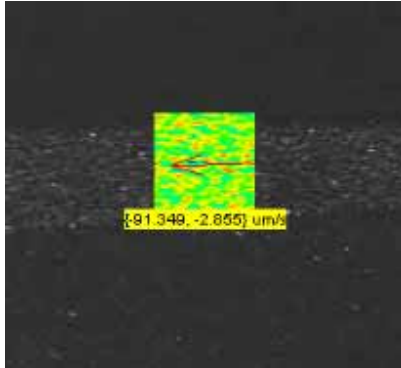
New probe showing correlation field, vector arrow and real time velocity as text

3. Repeat step 2 to create as many probes as desired.
4. Once created, the probes can be moved by clicking and holding the mouse button over the probe and dragging it to the desired location.
5. Click the + and – toolbar buttons to make the probes more or less transparent. The software will continue to calculate velocity at these locations even if you make the probes completely invisible.
6. To remove a probe, right click on it and choose Delete.

When you first create a probe uScope will experimentally determine the fastest FFT algorithm to use. This process may take up to 60 seconds to complete, at which point the software will begin calculating velocity at the location.

When you create a probe it will take on the properties of the last probe you altered. Once created, you can change each probe's properties individually.

7.1.3 Velocity Probe Properties

1. Double-click on a probe to open the **PIV Probe Properties** dialog box. You can also right-click on the probe and choose **Properties** to open the dialog box.
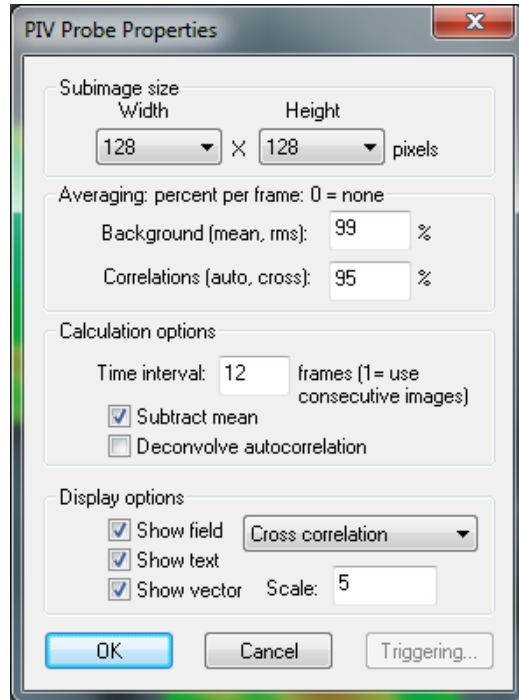
PIV Probe Properties dialog box

2. Select the **Width** and **Height** of the probe window, the area over which statistics will be calculated. Some guidelines for setting the probe window size are:
   a. Probes should typically be the same size in both x and y directions.
   b. Smaller probes require less processing power, so use smaller window sizes to run more probes simultaneously.
   c. Increasing the probe size will improve the signal-to-noise ratio; decreasing the size will increase spatial resolution.
   d. If the probe is located in a region of fast flow, the probe size must be large enough that the correlation does not fall beyond the window. The **Cross Correlation** field (see below) can be an aid in setting the size.

46

Note: If you cannot achieve sufficient signal-to-noise ratio with a required probe size you may need to adjust the illumination, particle feed, etc. If the probe is in an area of steady flow then increasing time averaging may also help.

3. The **Averaging percent per frame** values help separate the useful flow information from the background data by weighing the previous frames of data versus the current frame. The default values of 95% are acceptable for most flows. Some guidelines for setting the percentages are:
   a. The **Background (mean)** percentage determines how much of the image field is considered "background" based on its steady presence over multiple frames. Increasing this value increases the amount of information that is ignored, such as stuck particles. Check the **Subtract Mean** box (see below) to apply this calculation and remove the data.
   b. Decreasing the **Correlation** value improves time resolution; increasing the value improves noise control.

4. The **Calculations Options** control how the flow parameters are calculated:
   a. Enter **1** in the **Time interval** box to calculate cross correlation for every sequential pair of frames. Enter **2** to use every other frame, **3** for every third frame, etc. This option is useful for examining very slow flows.
   b. **Subtract Mean** subtracts the background (non moving) data from the flow calculations. The amount of data that will be subtracted is based on the **Background %** value (see above).

Note: If the probe is located in an area of very slow flow then subtracting the background could delete active particles.

   c. **Deconvolve Autocorrelation** is an advanced option for high precision measurements. This option deconvolves the cross correlation by the autocorrelation, which can remove

47

the effects of blur and particle size such that each particle is treated as a single point. It is most useful when the signal-to-noise ratio is extremely high.

5. The **Show Field** options control which data are displayed for each probe window:

   a. **Cross correlation** determines how far the particles move between frames (or between every few frames, based on the **Frames Skipped** option). This field is a good diagnostic tool to help you optimize experiment parameters. The red dot will move further from the center as the flow velocity increases. The dot should be small and well defined to achieve the most reliable measurements. If the flow is too fast the red dot will move outside of the window, and uScope will not be able to measure the velocity. In this case, increase the window size, which will improve the signal-to-noise ratio.

   b. **Autocorrelation** is an indicator of resolution. The mass at the center of the window will become sharper with smaller particles and better focus.

   c. **Mean** shows the data that is being subtracted as part of the Background (based upon the **Background %** described above). Showing the Mean can be helpful for highlighting stuck particles and other anomalies in the flow.

   d. **RMS** is an indicator of the amount of useful signal available for the calculations.

   e. **Show Text** turns on and off the text-based velocity display.

   f. **Show Vector** displays an arrow in the direction of the flow. The size of the arrow will change with velocity. You can also set the **Scale** to increase or decrease the arrow size.
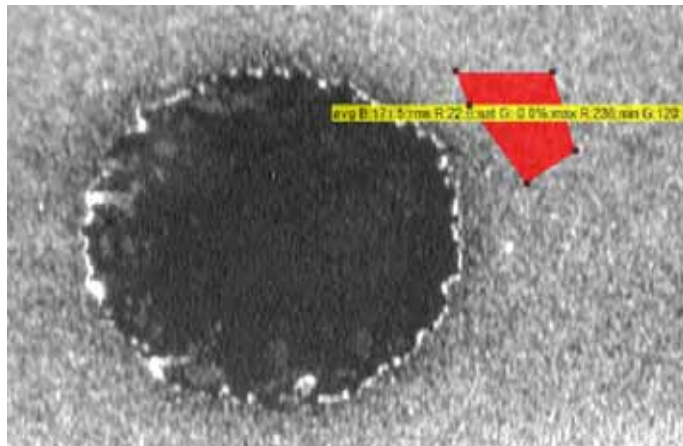
7.2   Intensity probes

Intensity or polygon probes are used to track the color spectrum or fluorescence intensity inside a defined region.

7.2.1 Creating intensity probes

To create a probe:

1. Click the **Intensity Probe** toolbar button to highlight the button.
2. With your mouse curser over the image, right click on the mouse and select New Probe. Click on the image to draw the outline of the probe. When the shape is defined double click the mouse to close the polygon. A probe such as the one below will appear.

New intensity probe

3. Repeat step 2 to create as many probes as desired.
4. Once created, the probes can be moved by clicking and holding the mouse button over the probe and dragging it to the desired location. The shape of the probe can also be modified by dragging the polygon points to create the desired shape.

7.2.2 Intensity Probe Properties

Intensity probe data displayed and recorded can be selected by selecting the probe (left click), then right click and select Properties. The following dialog box will appear.
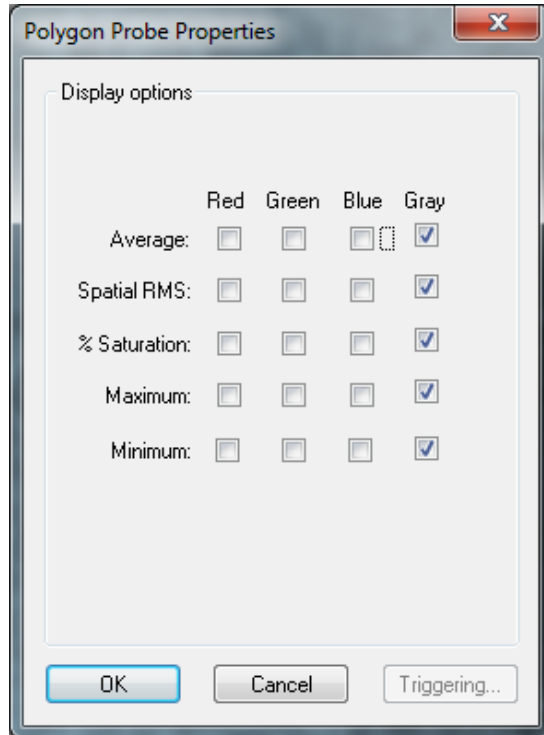
Polygon Probe Properties dialog box

For images from the B&W or EPI optics module only the Gray displays are applicable.

7.3   Recording probe data

Data can be recorded simultaneously from all probes. To record data:

1. Choose **File >Measurement File Naming** to select how the recorded data will be saved:
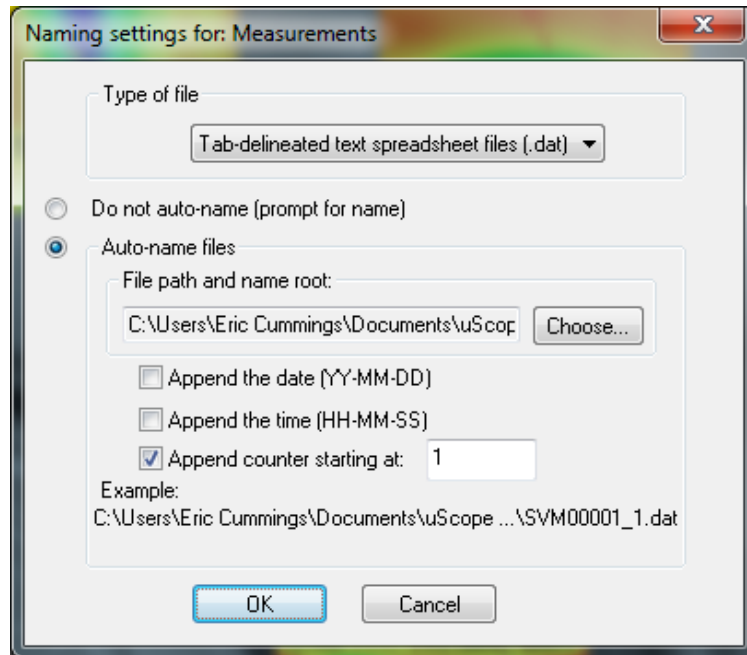
Naming settings for new measurements dialog box

  a. If you choose **Do not auto-name**, uScope will prompt you for a file name and location for each new recording.

  b. Choose **Auto-name files** to automatically name each recording. Check **Append the date**, **Append the time**, and/or **Append counter** to add these values to the new file names. An example of how the name will appear is shown at the bottom of the dialog box.

Note: You can currently only save the probes in the tab-delineated text spreadsheet file format.

2. To begin recording choose **File >Record**, or click the **Start/Stop** ▇ toolbar button. If Autonaming is selected, recording will begin immediately. Otherwise, recording will begin after you name the file and click **OK**.

3. To end recording, choose **File > Record** or click the **Start/Stop**

button again.

The PIV output file will include four columns for each probe: the X and Y locations of its centroid, measured from the upper left of the window, and the X and Y velocity at each point in time. The X/Y location columns will only have entries in the first row.

7.4 Saving probes

A set of probes can be saved to disk and recalled later:
1.   Arrange the probes and set their Properties.
2.   Choose **File >Save Probes As** to create a new probe file. Probe files are saved in the uScope file formate (*.usc).
3.   Select the name and location for the file and click **Save**.

To recall a stored set of probes choose **File > Open**.

8   TROUBLESHOOTING

8.1   Getting help

This guide is your main source for information on operating the SVM340 and the uScope software. The guide is also available on the uScope CD in PDF format for viewing with Adobe Acrobat.

Check the LabSmith web site (www.labsmith.com) for user manual updates, application notes and information to help you use the SVM340.

If you are unable to find the help you need, call the LabSmith technical support at (925) 292 5161 or send an e-mail to info@labsmith.com.
If you need support, please write down the serial number of your SVM340 (located on the bottom of the unit) and the version of the software you are using. To get the software version number, click Help>About uScope in the uScope main window.

8.2 No video signal

If the optics module is not focused on an object it sometimes appears that the you are not getting a signal. This is especially true with the EPI module (or a standard module when a filter is installed). The easiest way to see if you are in-fact getting a signal is to turn on and off the SVM, or unplug/replug the s-video cable. If you see a flash or any change to the image you are getting a signal. If you are not getting a signal, try the troubling shooting guide below.

- If you have multiple optics modules, try another one. If only one optics module is not working the problem is most likely with the optics module. If none of the optics modules are working the problem is most likely with the video card, video drivers, or video settings.
  - o Optics Module issues
    - ▪ Is the camera correctly installed in the SVM (top of camera box is securely against magnets on bottom of stage)?
    - ▪ Check connector pins on top of camera to ensure none of the pins are bent or broken.
  - o Video card/driver
    - ▪ Select Video>Video Capture Device to select the proper video capture device. If your video capture device is not listed:
      - o If you have installed an internal card, have you installed the drivers?
      - o If you have an external capture device:
        - ▪ Is the device plugged into a USB port?
        - ▪ Is the correct driver installed? Depending on your operating system, you may need to download the driver from the device manufacturer's website.
- Once you have chosen your video capture device, go to Video>Physical Input Connector and choose Video S Video In for the input connector.

- Ensure the SVideo cable is properly plugged into the back of the SVM and the video capture device or card
- Check the pins on both ends of the S Video cable to make sure none are broken or bent.

8.3 Video quality

- Fluorescence images: block room light
- Fast moving flows: use deinterlacing to eliminate "lines" across screen
- Steady image: use time averaging to improve signal to noise.

8.4 Dropped frames

The SVM uScope software uses a buffer as it is recording to minimize dropped frames. However, if the available RAM isn't sufficient you may still experience dropped frames.
Improving available RAM:

- Set the RAM buffer (should be > 500 MB): View>Video Buffer Settings
- Close anything running in the background that might be a resource hog: i.e., Windows Indexer, Antivirus scan, fetching and scanning emails, automated backup, etc.
- If you are using uScope Probes, close any that aren't necessary.
- Try running without interlacing and/or compression.
- Color Format: Running in true color (Video>Color Format>24-bit RGB) uses 3X more RAM compared to the B&W video options. If true color is not necessary, chose another option. Videos saved in true color will also be 3X the size of B&W videos.

The following table provides additional troubleshooting options:

Video and Image Quality

Settings	Darkfield (Fluorescence)			Brightfield		Phase Contrast	
Module (* indicates optimal module for application)	B&W	EPI*	Color	B&W	B&W	Color
Color Format	B&W or Fluor.	B&W or Fluor.	24-bit RGB	B&W or Fluor.	B&W or Fluor.	24-bit RGB
Analog Settings (Video >Brightness/ Contrast/ Hue/etc.)						
• Brightness	Adjust (typically low?)	Adjust (typically low?)	Adjust	Adjust (typically low?)	Adjust (typically low?)	Adjust
• Contrast	Adjust (typically high?)	Adjust (typically high?)	Adjust	Adjust (typically high?)	Adjust (typically high?)	Adjust
• Hue	No effect	No effect	Adjust	No effect	No effect	Adjust
• Saturation	Min	Min	Max	Min	Min	Max
• Sharpness	min	min	Adjust	min	min	adjust
Illuminator Settings						
• White light (typically channel C)	Off	Off	Adjust	adjust	adjust	Adjust
• Illuminator colored lights	Max	off	Adjust	adjust	adjust	Adjust
• EPI light (channel D/EPI)	n/a	max	n/a	n/a	n/a	n/a
• External/Room light	Off	off		off	on	

9 SPECIFICATIONS

Traverse

Range	*x*: 50mm, *y*: 75 mm, focus: 8mm
Resolution	*x* and *y*: 10 µm, focus (*z*): 1 µm

Sample stage

Dimensions	$X \times Y$: 140mm × 200mm
Opening	55 × 80 mm

Camera module

RS-170-BW	Analog, interlaced monochrome camera with 1/3" CCD 640 × 480 pixels, 30 frames/s
RS-170-C	Bayer-pattern analog color camera with 1/3" CCD 640 × 480 pixels, 30 frames/s
Objectives	10× plan 0.25/170
	4×
	20×
Illuminator modules	LED-B: 3 blue (center 460 nm, bandwidth 50 nm), one white bank
	LED-G: 3 green (center 560 nm, bandwidth 50 nm), one white bank
	LED-R: 3 red (center 660 nm, bandwidth 50 nm), one white
	LED-W: 4 white banks
	LED-RGBW: 1 red, 1 green, 1 blue, 1 white bank.
Inputs	4 programmable digital inputs, TTL level
Outputs	Composite analog video out
	S-video out
	3 programmable digital outputs TTL level
	4 external illuminator trigger/drivers, TTL level
Communication interface	Serial RS232, 9 pin D-sub connector.

Physical

Dimensions	W × L × H: 208 × 267 × 85 mm
Weight	2.8 kg
Power	90–240 VAC 47–63 Hz, 100 VA

Equipment List

Item	Qty	Use/Purpose
2" washer	1	Objective spacer – when trying to view items above the focal range of the SVM
Lubricant	1 bottle	Lubricate focus motor screws/bushings (located under Delrin stage) annually or as needed
Standoffs and washers	4	SVM is shipped with the standoffs/feet uninstalled to reduce the chance of damage during shipping. Install the standoffs in the threaded holes on the bottom of the SVM. Four rubber washers are included and can be put between the standoff and chassis to help level the SVM. The SVM chassis uses a sheet metal design to minimize weight and cost. The chassis can warp slightly during assembly or shipping. The kinematic design of the focus stage ensures that the slight warping will not affect the function of the SVM.
Filters and foam washers	As ordered	Schott glass filters are shipped with a foam washer. The filter is installed on the optics module by removing the objective and placing the filter in the recessed area in the objective mount. The foam washer is used to hold the filter in place when the objective is reinstalled. Note: Take care not to introduce dust into the optics module when the objective is removed. If the objective will be left off the optics module, place something over the objective mount (i.e. a piece of tape) to prevent dust from entering the module.