#### Pol-Acquisition, OpenPolScope software for Micro-Manager

Last Updated: April 11, 2013; Revision 1.07

This manual was prepared based on Pol-Acquisition software version 2.0 and Birefringence Processing enabled.

Other versions of this manual are being prepared with Dichroism Processing and with Fluorescence Polarization Processing enabled.

Copyright, license, and warranty/disclaimer statements at end of document.

## **Table of Contents**

Before Installation	2
Overview	4
Installation	5
Starting the Pol-Acquisition plugin	6
The Pol-Acquisition window	6
A Walk-Through of Basic Operations	7
Calibrating the variable retarder settings	7
Acquiring birefringence images	9
Calibration of calculated retardance and orientation values	10
Mapping of calculated retardance and orientation values into 8-bit and 16-bit images	10
Overview of Functionality and Interface Tabs	11
Data tab	11
VariLC tab	12
Controls	12
Calibration Options	13
Save & Load	
Options	
Parameters tab	
Birefringence tab	
Processing tab.	
Uptions tab	
Logger tab.	
Acquisition Buttons	
нер	
Report Tool	
ChangeLog	
About	
Appendix: A	26
Multi-Mode Acquisition	
Appendix: B	31
Calibrating the OpenPolScope for measurement of slow axis of birefringence using chee	k cells
	31
Copyright, license, warranty/disclaimer	36

#### **Before Installation**

*OpenPolScope* plugins come in two flavors, which are installed in different plugin folders inside the Micro-Manager folder:

- 1. Pol-Acquisition plugin; after its installation into the MMplugins folder, this plugin appears in the Micro-Manager Plugins menu. It is used to acquire PolScope images, applying PolScope algorithms and controlling the *OpenPolScope* hardware attached to the microscope.
- 2. Pol-Analyzer plugin; after its installation into the plugins folder, this plugin appears in the ImageJ Plugins menu, providing functions to view and analyze PolScope data that have been previously acquired.

The *OpenPolScope* software installer installs both plugins.

#### Software requirements:

- 1. Micro-Manager (tested with release 1.4.13, subsequent changes in Micro-Manager code might result in unexpected behavior)
- 2. *OpenPolScope* software Installer for Windows XP, 7, and Vista: LCPolScopeSetup.exe Installer for Mac: LCPolScopeSetup.pkg

#### **OpenPolScope** software components:

Micro-Manager Plugins menu: Pol-Acquisition, FrameAverager, TopFrame

Image J Plugins menu PolScope: Pol-Analyzer; Orientation-Lines

PolScope Legacy: RatioAzimPolStackToRGB\_V2; ROI\_Averages\_With\_Lines\_V1; StackAverage\_

Java Console

#### Minimum and recommended system requirements

- Hardware:
  - 1. *OpenPolScope* hardware, consisting of liquid-crystal (LC) universal compensator and VariLC or equivalent electronic controller.
  - 2. Camera, supported by Micro-Manager
  - 3. Computer recommended 1.5GHz CPU dual core, 64-bit
  - 4. 2 GB RAM (recommended 4 GB RAM)
  - 5. 10 MB Hard Disk Space (additional space required for UserData)

#### • Software:

- Windows XP, Windows 7, Mac (32/64 bit) (*OpenPolScope* software is currently not tested on Mac)
- 2. Java 1.6 update 31

**Note:** It is recommended to increase the ImageJ memory (Edit>Options>Memory...) to a minimum of 1024MB or more for smooth camera operations.

#### Guide to abbreviations

VariLC Digital controller for liquid crystal devices. The controller, which might have a name different from VariLC, is connected to the computer through a serial or USB cable.

File naming	SM_2012_0904_0213_5	Prefix:
convention		SM – Sample
	<pre>Prefix_Year_MonthDate_HourMin_Suffix</pre>	BG – Background
		SMS - Sample Series
		(Multi-Dimensional)

## **OpenPolScope data directory structure**

Pol-Acquisition organizes the image data according to the following directory tree:

```
<LCPolScopeData> ------ (user chosen top directory)
```

```
|
<UserName1> ----- (contains directories of a single user/project)
    |
        <SessionDate1> --- (contains image and metadata files of a day's session)
        |
        <SessionDate2>
<UserName2> ------ (contains directories of another user/project)
        |
        <SessionDate1> --- (contains image and metadata files of a day's session)
        |
        <SessionDate2>
```

#### **Overview**

The following schematic summarizes the hardware configuration typically required for birefringence imaging with the OpenPolScope:



raw LC-PolScope images (intensity images)

computed PolScope images

The optical design (left) builds on the traditional polarized light microscope with the conventional compensator replaced by two variable retarders LC-A and LC-B. The polarization analyzer passes circularly polarized light and is typically built from a linear polarizer and a quarter wave plate. Images of the specimen (top row, aster isolated from surf clam egg) are captured at five predetermined retarder settings, which cause the specimen to be illuminated with circularly polarized light (1st, left most image) and with elliptically polarized light of different axis orientations (2nd to 5th image). The retarder settings are computer controlled through the retarder controller, also called VariLC. Based on the raw PolScope images, the computer calculates the retardance image and the slow axis orientation or azimuth image using specific algorithms.

### Installation

For setting up the *OpenPolScope* hardware, the user needs to be familiar with Micro-Manager and its Hardware Configuration Wizard (in Tools menu). The wizard is used to add the camera and VariLC hardware to the Micro-Manager configuration file (xxx.cfg). These additions are necessary before the Pol-Acquisition plugin can be used. Once the VariLC has been added, its properties have values similar to the ones seen in the image below . The Port Properties required for communicating with the VariLC are the default values for COM-port communication. **For the Abrio**, the baud rate needs to be set to 115200, instead of 9600, the total number of LCs to 3, and active LCs to 2. One can use the Property Browser in the tools menu to test the communication with the VariLC.

🛃 Sys	tem: C:\Micro-Ma	nager-1.4\QCam_VLO	_Com3_150ms_Demo	Stage.cfg			×		
File T	ools Plugins Helr	p							
	Snap Came	era settings	Configuration	settings		S	Save	Charles and the second	
H	lardware Configura	ation Wizard						La V	x
	Step 2 of 6: Add or	remove devices							
	Installed Devic	es:			_				
	Name	Adapter Library	Description	Charles	Edit.		Adding or J	Removing Devices	
Plea	Core	MMCore/Default	Core controller	Status	Derinher				
RO	QCamera	QCamera/QCam	QImaging universal	I OK	- Peripriere	als	1. The list	t above displays all of	
	VariLC	VariLC/VariLC	VariLC	ОК	Remov	ove	the dev	vices that will be handled	
Ima		1			1		hu Mic	-Manager in this	
-			🛃 Device: VariLC   I	Library: VariLC				ition file.	
C			Intel Varil C					e making a new	E
			Laber varies					tion file for the first	
197			Teitislization Propert					ise visit the	
			Initialization rope.se	25 Dranarky	_	and a		anager website	
			Device	Property		Value		icro-manager.org) and	
H			VariLC Varil C	Port	tive LCs	2 COM3		er Devices to find	
			VariLC	Total Number	r of LCs	2		ms for setting up all	
	Available Devic	ces: list by vend	VariLC	Total Number	of Palette Ele	5		ices.	
	The source out	Jgc						begin adding new	
	Thoriabs							whenever you're ready	
	ThorlabsF	FilterWheel						Id' button). II you	
	ThorlabsS	5C10			-			e help with deciding	
	🗊 📜 Tofra					C		vices to add, mgningin	
	TwainCam	nera	Port Properties (RS 2	232 settings)		L	Scan	ar device and click	
	VariLC		-	Property	_			ting a device you can	
	VariL(	.C   VariLC	Device	Property		Value		allow the wizard to	
	😥 🕕 📗 Vincent		COM3 COM3	BaudRate	ut	9600	/	these devices	
	XCite 120P	PC_Exacte	COM3	DelayBetwee	nCharsMs	0.0000		ally (recommended)	
	XCiteLeu Xcite		COM3	Handshaking	6	Off		all of the subdevices	
	Yokogawa	a	COM3	Parity		None		(how all')	-
	E ZeissCAN	i	COM3	StopBits		1		Next >	
			COMS	Verbose	_	1		Sack Next >	
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							Cancer		
1.	Provide and the second							9	
П.	Configur	A MC and	-						52
1	lardware Conligue	ation Wizaru		2					
	Step 4 of 6: Set del	lays for devices without	synchronization capabil	lities					
					_				
				1.0000000			Catting Dov	Dalarre	Â
	Name	A	dapter	Delay [ms]			Setting Devi	ice Delays	
Plea	DWheel	DV	Wheel	0.0			1		
RO	DStateDevice	DS	StateDevice	0.0			<ul> <li>Some d</li> </ul>	levices will execute a	
	VariLC	Va	ariLC	150.0			comma	and but don't signal to	
							Micro-'	Manager when	

**Note**: The VariLC is synchronized with the camera or other hardware through a delay time setting, since the VariLC controller does not provide a state change callback. The delay time to be used depends on the physical properties of the liquid-crystal devices. A typical delay time is 100 ms, but can be shorter. **For Abrio** use 100 ms.

Before using the system, we recommend that you go through the process described in the section "A Walk-Through of Basic Operations" to configure and calibrate the system correctly.

#### **Starting the Pol-Acquisition plugin**

System: C:\/	🛃 System: C:\Micro-Manager-1.4\QCam_VLC_Com3_150ms_DemoStage.cfg						
File Tools Plu	igins Help						
Sn.	Clojure editor	Configuration settings		Save			
Liv	ASI CRISP Control	Group	Preset				
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Multi-D	Frame Averager	System	Startup				
Refre	Localization Microscopy	se					
Please cite I	Image Flipper	Je					
ROI	Multi-Andor Control	IS					
	Pixel Calibrator	Group: + -	Edit Preset: +	Edit			
Image info (I	Projector	range: 8 bits, 0nm/pix, Z=0.00um, XY=(-0.	.00,-0.00)um				
Contract	Pol-Acquisition						
Contrast	Live Replay PolSc	ope Acquisition	Tel III				
Scale E	Slide Explorer	Sync channels	Slow hist				
Displa	SplitView	Autostretch ignore % 2	2 📩 🗌 Log hist				
	Stage Control	Stage Control					

The Pol-Acquisition plugin can be accessed from the Micro-Manager Plugins menu.

If the Pol-Acquisition plugin is accessed without the VariLC ever being connected to the computer and configured as a hardware component in Micro-Manager, an information prompt will come up "VariLC not detected" and VariLC functions are disabled.

#### The Pol-Acquisition window

Pol-Acquisition (MicroManager)
Help
User: Rudolf Oldenbourg Session: 2013_03_28_Birefringence Acquire Birefringence
SM BG Series
Acquisition Comments
Preferences saved.
Data Location
Data Location C:\Users\Amitabh\Desktop\LCPolScopeData
User: Inductional User:
Session: 2013_03_28_Birefringence  Create New
Session Description: Birefringence data (spermatocyte)
Set Default to 'Save' Acquired Images

After correctly configuring the hardware, the Pol-Acquisition plugin can be accessed. A window opens that contains action buttons, a progress bar, and several tabs that let the user set measurement parameters, settings, options, data locations, etc.

## A Walk-Through of Basic Operations

While performing this sequence, you may wish to refer to the section "Overview of Functionality and Settings Tabs"

#### Start the Micro-Manager software:

After the camera and VariLC controller have been turned on, you may launch the Micro-Manager software by double-clicking the MM icon on the Windows desktop. In the startup query window, choose the MM config file that was prepared beforehand (see **Installation**) and includes the camera and VariLC controller.

#### Setup your sample:

Prepare your microscope for Koehler illumination. Place your sample in the field-ofview, select appropriate objective, and direct the majority of the light to the camera. Focus on your sample using the live camera window. For calibration, move the sample sideways out of the live camera window to image a clear, non-birefringent sample area, also called background

#### Set acquisition and processing algorithms

Select the Processing tab in the Pol-Acquisition window and set Birefringence as the acquisition and processing algorithm. For starters, deselect pre- and post-processing algorithm(s).

#### A Note on Setting the Swing and Retardance Ceiling

The swing setting should be adjusted based on the sample/specimen you wish to analyze.

Thin biological samples typically require a swing of 0.03 and a retardance ceiling of 5 nm or higher.

Thicker samples or samples containing crystals should have a higher swing setting (usually 0.1).

#### Calibrating the variable retarder settings

At the beginning of a session, and sometimes during a session, the five liquid crystal settings need be calibrated. Setting 0 generates circularly polarized light and is called extinction setting for which LC-A is nominally 0.25 (quarter wave) and LC-B is 0.5 (half wave). Settings 1 through 4 generate elliptically polarized light and LC-A and -B deviate from the extinction values by adding or subtracting a swing value. The automatic calibration routine optimizes the extinction and the elliptical settings, based on the swing value chosen by the user.

The system should be calibrated while observing the slide near the area where your sample/specimen of interest is located, as background birefringence can vary across the slide.

Before starting calibration, make a ROI selection in the camera window that identifies a specimen area devoid of specimen birefringence.

Identify calibration parameters:

- 1. Select the VariLC tab in the Pol-Acquisition Window.
- 2. Identify appropriate swing value. If you are unsure of this value, you may begin by entering a median value of 0.1. Once the actual range of retardance

values in your sample are known, you may adjust this number for improved performance.

- 3. Check your lamp power and camera exposure time by selecting the "Check Intensities" tab. If the intensity readings are above 210, lower the lamp power and/or reduce exposure time.
- 4. Set Black Level. Block light from reaching the CCD and acquire an image. Determine the average pixel value and enter it in the Black Level. Recommended is a value between 3 and 7 for 8-bit images. If the value is unacceptable, change the camera offset setting.
- 5. Press Calibrate. Calibration will take roughly a minute to complete.
- 6. Optimize exposure based upon calibrated LC settings.

With optimized exposure, the numbers from your results should be within the following ranges (assuming 8-bit images with pixel values between 0 and 255):

LC Setting Intensity

0	10 to 80
1	80 to 210
2	80 to 210, equal to setting 2, +/- 3 cts
3	80 to 210, equal to setting 2, +/- 3 cts
4	80 to 210, equal to setting 2, +/- 5 cts

If the numbers from your results fall significantly out of the ranges shown above, you will have to perform the calibration sequence again. Check the microscope to make sure that all *OpenPolScope* components are present *and that there is nothing in the optical path that may interfere with proper operation*. Be sure to remove common accessories such as filter cubes or linear polarizers that may have been left in place by a previous user.

During calibration, if three successive measurements are too bright, the calibration will stop and display a warning.

You may adjust the lamp and repeat the calibration sequence until all the values are in the desired ranges. Nevertheless, even if the Average Gray values of Setting 2 through 5 are as low as 40 or as high as 230, the *OpenPolScope* will still function, though not optimally.

The ratio of Setting 2 to Setting 1 is an indicator of the retardance measurement signal-tonoise ratio. Bigger ratios are better. For large retardance values, which usually involve a retardance swing of 0.2, the ratio is 10 to 1 or better. For small retardance values, which usually involve a retardance swing of 0.03, the ratio is typically 2 to 1 or better.

For microscope applications, the diameter of the aperture diaphragm in the condenser has a significant impact on this ratio, and thus the retardance signal-to-noise ratio. If the aperture is decreased, this ratio will go up. However, signal will go down and the exposure time will increase. Of course, the adjustment of the condenser aperture will also impact spatial resolution and depth-of-focus.

The Extinction number is another indicator of the quality of the polarization optical train. An Extinction of at least 100 should be achieved, more routine is 200 and better. The optical setup will be more sensitive the higher the Extinction.

Do note that if you change the swing value, you will need to recalibrate.

It is advisable to re-calibrate whenever you change the sample/specimen slide.

#### Acquiring birefringence images

Set acquisition parameters:

Select the Parameters tab in the Pol-Acquisition window. Choose whether or not a mirror is present in the optical path, the Black Level and the use of frame averaging are set correctly. Please note that recalculation after image acquisition is possible should the settings initially be incorrect (refer to Pol-Analyzer manual).

Set pre- and post-processing algorithms

Select the Processing tab in the Pol-Acquisition window and select the desired preand post-processing algorithms.

Set birefringence parameters

Select the Birefringence tab in the Pol-Acquisition window and designate an appropriate retardance ceiling for computed retardance images. For biological samples, this is often in the 5 – 10 nanometer range.

#### Acquire a background image

If the retardance values in the sample are less than 20 nm, as is often found in biological images, we recommend that you take a background image. Background images are taken without a sample in the field-of-view, but with the microscope slide in place. These images are used to compensate for the birefringence of the microscope optics themselves.

Position your sample so that the live camera window is free of sample and debris. Defocusing slightly can help to reduce the visibility of particles in the area of the slide where the background is acquired.

Proceed to take a background stack by clicking the BG acquisition button from the top of the Pol-Acquisition window. The new background data will be saved to disk if "Save to Disk Acquired Images" is checked in the Data tab.

The name of the newly acquired background stack will appear in the Background field of the Parameters tab and will be used for background correction of the samples subsequently acquired.

It is advisable to acquire an additional background image at the end of a time series, as the variable retarders may drift and corrections can be applied later to compensate for this.

#### Acquire a sample image

Position and focus on your sample in the Live Camera window. Proceed to acquire an image stack by clicking the SM (sample) acquisition button from the Pol-Acquisition window. The newly acquired image will appear and automatically be saved to disk if "Save Acquired Images" is checked in the Data tab.

The resulting sample image should appear in a new viewer window with background correction applied. If no background stack is specified, the sample retardance and orientation values are computed without background correction.

Once you have successfully acquired a sample image, you are ready to perform

Multidimensional acquisitions (e.g. z-slices, time series, etc.)

#### Calibration of calculated retardance and orientation values

The calculated retardance and orientation values are best calibrated against a sample for which the retardance and slow axis orientation values are known. This is particularly important for the slow axis orientation, which depends on physical parameters of the optical setup, including the orientation of the liquid crystal compensator and of the camera.

The **retardance** values are already calibrated by virtue of the calibration of the liquid crystal devices. The manufacturer of the LCs of the Oldenbourg Lab included a calibration table in the VariLC controller, which allows the setting of an LC device to an absolute retardance, usually expressed as a fraction of the wavelength. Therefore, the swing value is given in absolute retardance, and the specimen retardance in each image pixel is calculated in absolute retardance. (Note that the retardance of the LC-settings and the swing are given in fraction of wavelength, while the retardance of the sample is given in nm)

The **orientation** of the slow axis needs to be calibrated using a specimen with known slow axis orientation. The calibration involves the correct setting of the orientation reference angle (see "Birefringence tab") and of the mirror setting (see "Parameters tab"). A number of test specimens can be used for this purpose. Biological specimens such as living (or fixed) cells with stress fibers or dividing cells with a living spindle or well preserved spindle microtubules are well suited, because the morphologically distinct fibers are birefringent with a slow axis orientation parallel to the fiber axis. Also collagen fibers have a slow axis that is oriented parallel to the fiber axis.

A simple and accurate calibration sample can be made with a thin glass fiber (e.g. soda lime glass, diameter  $\sim 100 \ \mu$ m) that is taped to a microscope slide. When taping the fiber down on both ends, induce a slight bend in the fiber. The bend causes stress birefringence whose slow axis is oriented in a predictable fashion with respect to the fiber axis. The birefringent layer on the outside of the bend, where the soda lime glass is stretched, has its slow axis parallel to the fiber axis, while the birefringence in the inside layer, where the glass is compressed, has its slow axis perpendicular to the fiber axis. For best imaging results, the fiber can be imbibed in immersion oil to remove the cylindrical lens effect of a fiber that is surrounded by air. (refer Appendix B for alternate method using cheek cells)

#### Mapping of calculated retardance and orientation values into 8-bit and 16-bit images

The calculated images in channel 1 and 2 represent the measured retardance (channel 1) and orientation of the slow axis (channel 2) values in every pixel of the sample or background image. As all images associated with a PolScope stack are either 8-bit or 16-bit images, including channel 1 and 2, the calculated retardance and orientation values are mapped into either 8-bit or 16-bit integers.

The computed **retardance** values are linearly mapped from 0 to the retardance ceiling value into integer values from 0 to 255 (8-bit) or 0 to 65,535 (16-bit). Retardance values that are computed to be higher than the ceiling value are converted to 255 or 65,535, respectively.

A pixel value in channel 1 can be converted into a retardance value by the following formula:

8-bit image:	retardance = $\frac{\text{pixel_value}}{254}$ · retardance_ceiling
12-bit image:	retardance = $\frac{\text{pixel_value}}{16,382}$ · retardance_ceiling
16-bit image:	retardance = $\frac{\text{pixel}_\text{value}}{65,534}$ · retardance_ceiling

The highest possible pixel value (255 for 8-bit, 16,383 for 12-bit, and 65,535 for 16-bit images) is reserved for indicating any value higher than the retardance\_ceiling.

The computed **orientation** values range between 0 and 180° and are stored as integer values from 0 to 180 (8-bit), 0 to 1800 (12-bit), or 0 to 18000 (12- and 16-bit). Hence, an orientation angle stored in channel 2 of a 16-bit image is equal to the pixel value divided by 100.

## **Overview of Functionality and Interface Tabs**

#### Data tab



#### VariLC tab

This section applies when using VariLC or Abrio as a liquid crystal device controller.

#### Controls

Dat	a VariLC	Parameters	Birefringence	Processing	Options	Logger	The VariLC tab has controls for
							the LC universal compensator
	Swing:	0.03	fraction		Rese	t LC	with 2 liquid crystals, LC-A and
N	avelength:	546.0	nm		Exercis	se LC	LC-B.
				_			Swing: Sets the nominal
С	ontrols C	alibration Opti	ons Save & Lo	ad Options			retardance bias (as a fraction of
		L	_C-A	LC-B	Inten	sity	the wavelength) for the elliptical
	Current	t 0.	286 🌻 🔹	0.422 🌲	34.0	08	settings of the liquid crystals. Default is 0.03.
	Defaults	•	Calibrate		Cheo	ck	Wavelength: Sets the wavelength
	Setting (	0	.256 🌲 🤇 (	).422 🚔	0.00	00	that will be used when setting the Retardance values Usually equal
	Setting 1	I 0	.286 🌲 (	).422 🌲	34.0	08	to center wavelength of light used
	Setting 2	2 0	.256 🌲 (	).457 🌲	0.00	00	to illuminate specimen. Default is
	Setting 3	3 0	.256 🌲	0.39 🌲	0.00	00	546 nm.
	Setting 4	4 0	.223 🔶 🤇 (	).422 🚔	0.0	00	Reset LC: Resets the Error light on the VariLC controller.
[	0.001	0.001	▼ Extin	ction Ratio	0.0	0	Occasionally, this light might come on during the calibration process, without consequence.
							Exercise LC: Cycles the LCs

between high/low values to assure consistent settings. Click if LCs have been powered off for a long time and intensities measured for separate settings are not reproducible. (Click Check several times and watch intensity values.)

<u>LC-A and LC-B</u> (columns): retardance values of LCs as fraction of wavelength; can be changed by either typing value or clicking spinner wheel.

<u>Intensity</u> (column): intensity recorded in camera ROI; is updated each time LC retardance is changed.

Current (row): current retardance values of LCs

The remaining buttons and fields in the lower half of the VariLC tab affect the five settings used for *OpenPolScope* imaging:

<u>Defaults:</u> Populates the Settings with the nominal LC retardance values. The Settings are not calibrated

<u>Setting</u>: Setting 0 is also referred to as the Extinction Setting. The other four Settings are the swing applied settings that are calibrated during the calibration procedure. Clicking a Setting button, sets the LCs to the corresponding retardance values.

<u>Check:</u> Measures intensities for Settings 0 through 4 and calculates the extinction coefficient.

<u>Calibrate:</u> Calibrates the five LC settings using intensity readings from the camera ROI.

Calibrating the 'Extinction' Setting 0 searches for an intensity minimum by varying the retardance values of LC-A and LC-B. The search starts from the values shown in the Current Setting. For best results, start from a setting that is close to the minimum.

Once the Extinction values are found, the swing is applied to the next settings and calibrated such that their intensities are equal.

Before starting calibration, make a ROI selection in the camera window that identifies a specimen area devoid of specimen birefringence. Typically, calibration is done with the camera showing a background region in the specimen and is immediately followed by recording a Background stack.

Extinction field shows the extrapolated extinction coefficient. The extinction coefficient characterizes the quality of the polarization optical train. The extinction is defined as the ratio of the recorded intensity when the universal compensator is set to maximum transmission divided by the intensity when the compensator is set for minimum transmission (extinction). is measured directly (Setting 0), while the maximum  $I_{ext}$ 

transmission is extrapolated from Setting 1, using the measured intensity  ${\mbox{ and } I_1}$ 

extrapolating to a virtual setting that would cause maximum transmission:

$$Extinction = \frac{I_1 + I_2 + I_3 + I_4 - 4I_{ext}}{4 \cdot (I_{ext} - BlackLevel) \cdot \sin^2(Swing \cdot \pi)}$$

**Note**: The Calibration routine can be tweaked by using the Brent Optimizer Accuracy and Low/High range limits of the VariLC from the Options panel.

**Note**: It is recommended to change the VariLC settings through this tab rather than Micro-Manager's Device/Property Browser.

#### **Calibration Options**

Controls Calibration Options Save & Load Options	Plot Intensities: Plot the Intensity profile during Calibration.
Plot Intensities     Plot Retardance Values     Use Sequence Acquisition for Calibration	<u>Plot Retardance Value</u> : Plot the Retardance Value profile during Calibration.
Extinction Search Coarse Factor	Use Sequence Acquisition for Calibration: A faster mode for Calibration and is required enabled for certain cameras (eg. Andor). If
Brent Optimizer Absolute Accuracy Coarse 0.0100000	Calibration using this mode causes error during Calibration you may disable it. De- fault is enabled.
Brent Optimizer Absolute Accuracy Fine 0.0000100	Extinction Search: When performing a Cal- ibration this defines the search range from the current value. The final search range is the multiple of swing defined. Default is 4
	for Coarse and 2 for Fine.

The Brent Optimizer defines the tolerance or accuracy of the search. Default is 0.01 for Coarse and 0.00001 for Fine.

Thus with a swing of 0.03 and a factor of 4. The LC will scan a range of +/- 0.12 from the current LC value with its respective tolerance.

#### Save & Load

ontrols Calibration Options Save & Load Option	ns Save Var	<u>iLC Settin</u>
Save Varil C Settings	the curre	ent LC sett
Filename:	with con	iments or
	the LC se	(.poiset).
Save Load save palette set	tings in text file in the IJ_	Prefs.txt f
Comments:		

A user can save gs to a text file ad a saved setis is optional, as so routinely saved

#### Ontions

Controls Calibration Options Save & Load Options	<u>Device</u> :
Device Serial No.: V 10.00 2 400.0 700.0 70017 VariLC Range Low 0.0 High 0.84 Vuse VariLC Mapped Values	VariLC Range: These upper and lower limits need to be determined for the li- quid crystal devices installed and need to be set once during installation.
Export Mapped Values Sheet         Import Mapped Values Sheet           Only values in green should be edited in the Excel sheet	<u>Use VariLC Mapped Values</u> : If checked, a device specific calibration table for li- quid crystal settings is used. Default is enabled (checked). If unchecked, num-

bers in LC settings are sent directly to VariLC controller.

Export/Import Mapped Values Sheet: The currently used Mapped values can be exported as an excel file. A default template is exported in the case of Non-Profiled devices. This is particularly useful for Non-Profiled LC Devices where the default values can be edited on the excel file and imported back in the software. Imported values are not retained if Micro-Manager is restarted and the excel file needs to be imported again.

#### Parameters tab

ata VariLC Parameters Birefringence Processing Options Logger	The Parameters tab includes
Background: No Background	acquisition and are saved in the image metadata.
Image-Path Mirror: No  Black Level: 4.0	<u>Background</u> : The selected background that is used for background correction when
Averaging Sample 8 Background 8	computing the specimen retardance and slow axis orientation.
	orientation.

Note: Background data can be

stored in a file located on disk or in an open window. Background data should be recorded at least once at the beginning of a session.

<u>Image-Path Mirror</u>: Used for an Inverted microscope or if there is a mirror in the optical path. Default is No.

Setting the mirror parameter inappropriately leads to incorrectly computed orientation values. Retardance values are not affected by the mirror setting.

<u>Black Level</u>: The Black Level (also called baseline) is the average pixel value when the camera is not illuminated. The black level primarily depends on the camera offset setting.

Averaging is available if the 'FrameAverager' plugin has been installed in the Micro-Manager plugins folder (appears in the MM plugins folder). Averaging generates raw image data by averaging N camera frames. Averaging reduces image noise at the expense of time resolution. Averaging more than 8 frames is usually not effective. Default is Sample disabled and Background enabled at 8.

#### **Birefringence tab**

Data     VariLC     Parameters     Birefringence     Processing     Options       Retardance Ceiling:     20.0     nm     Image: Constraint of the second sec	The main 'Processing Mode' selected in the Processing panel (here Birefringence) will be displayed as an additional tab. Its panel will contain options only applicable when this Mode is selected.
Use 4-Frame Acquisition instead of 5-Frame	Retardance Ceiling: Sets the value of the retardance ceiling that is used for storing the retardance values in its image channel (usually first channel). For further explanations, see section "Mapping of calculated retardance and orientation values into 8-bit or 16-bit images"
	Orientation Reference: Sets the orientation reference that is used for processing the slow axis orientation image.

<u>Use 4-Frame Acquisition...</u>: Option to acquire only 4 frames instead of the default 5 frames, dropping the acquisition of the raw image for Setting 4.

<u>Use 4-Frame Processing...</u>: Option to use only 4 frames for processing instead of the default 5 frames.

#### Processing tab

ata	V	/ariLC	Parameters	Birefringe	ence	Processing	Options	Logger	
#		Pre-P	rocessing	Enabled	Des	cription			Options
	0	Ratioin	Ig		Ratio	oing use a RO	I selection	to scal	
#		Proce	essing Mode	Enabled	Des	cription			
#	0	Proce Birefri	essing Mode	Enabled	Des Calc	cription ulates Birefrin	igence, Oi	rientation	using 🔺
#	0	Proce Birefri Demo1	essing Mode ngence TEST Plugin	Enabled	Des Calc DEM	cription ulates Birefrin O-TEST Pol-pl	igence, Oi lugin using	rientation 5 frame:	using 🔺
#	0 1 2	Proce Birefri Demo Dichro	essing Mode ngence TEST Plugin ism	Enabled	Des Calc DEM Dich	cription ulates Birefrin O-TEST Pol-pl roism calculat	igence, Oi lugin using tes the opt	rientation 5 frames tical aniso	using A s and ptropy E
#	0 1 2 3	Proce Birefri Demo Dichro Fluore	essing Mode ngence TEST Plugin ism scence	Enabled	Des Calc DEM Dich Fluo	cription ulates Birefrin O-TEST Pol-pl roism calculat rescence calc	igence, Or lugin using tes the opt culates Flu	rientation ) 5 frame: tical anisc	using s and ptropy
#	0 1 2 3 4	Proce Birefri Demo Dichro Fluore RT_Po	essing Mode ngence TEST Plugin ism scence	Enabled	Des Calc DEM Dich Fluo Real	cription ulates Birefrin O-TEST Pol-pl roism calculat rescence calc Time (Quad)	igence, Or lugin using tes the opt culates Flu Fluoresce	rientation ) 5 frames tical aniso torescent nce calcu	using s and btropy ce-Pol ulates
#	0 1 2 3 4	Proce Birefri Demol Dichro Fluore RT_Po	essing Mode ngence TEST Plugin ism scence	Enabled	Des Calc DEM Dich Fluo Real	cription ulates Birefrin O-TEST Pol-pl roism calculat rescence calc Time (Quad)	igence, Oi lugin using tes the opt culates Flu Fluoresce	rientation ) 5 frame: tical anisc iorescenc nce calcu	using A s and otropy E ce-Pol Jlates T
#	0 1 2 3 4	Proce Birefri Demo Dichro Fluore RT_Po	essing Mode ngence TEST Plugin ism scence I	Enabled	Des Calc DEM Dich Fluo Real	acription ulates Birefrin O-TEST Pol-pl roism calculat rescence calc Time (Quad)	igence, Or lugin using tes the opt culates Flu Fluoresce	rientation ) 5 frame: tical aniso torescent nce calcu	using A s and otropy E ce-Pol Jlates T
#	0 1 2 3 4	Proce Birefri Demo Dichro Fluore RT_Po Post-	essing Mode ngence TEST Plugin ism scence I Processing	Enabled © © © Enabled	Des Calc DEM Dich Fluo Real	cription ulates Birefrin O-TEST Pol-pi roism calculat rescence calc Time (Quad)	igence, Or lugin using tes the opt culates Flu Fluoresce	rientation ) 5 frame: tical aniso ioresceno nce calcu	using s and s and thropy ce-Pol Jlates T

The Processing tab is divided into 3 main segments.

*Pre-Processing*: This applies some modifications to the raw image data before they are sent to the main processor. An example is Ratioing in which the pixel values of the raw sample images are multiplied by a correction factor calculated based on the background images. Multiple *Preprocessors* can be selected.

**Note:** The raw image data that are stored in the raw image channels are never altered during any processing steps, including

preprocessing and post-processing.

*Processing Mode*: This is the main processor, which controls the acquisition and processing algorithms used. It defines the number of raw images acquired; the number of computed images, which are also called virtual channels; the computation algorithm generating the virtual channel images; along with other parameters. Only one *Processor* can be selected. A Processor has its own options tab (Birefringence).

*Post-Processing*: This applies some post-processing correction to the images. It can also be used to generate additional images. Multiple *Post-processors* can be selected.

**Note**: All the *OpenPolScope* processors that are currently installed will be displayed in this tab.

Note: Acquisition cannot be performed without selection of a Processor plugin.

**Note**: *Pre-/Post-Processor* may or may not have additional options that are indicated by a button.

#### **Options tab**

Data VariLC Parameters Birefringence Processing Options Logger	Multi Mode Acquisition:
Multi-Mode Acquisition Ask to merge with preconfigured Group Always ask for Pol related Hardware Settings on Merge	For merging PolScope acquisition with another mode of imaging, such as fluorescence or bright field, defined as acquisition Group.
Display	(refer Appendix A for more details) Ask to merge with preconfigured
Processing  Enable On-the-fly Processing during Acquisition  Parallelize Processing	<i>Group:</i> allows merging with an acquisition Group that has been previously defined by a user.



*Always ask for PolScope related Hardware Settings on Merge:* If the merge option has been used previously the fields can be populated using the stored values.

#### Display:

*Show Computed Image During Acquisition*: During series acquisition, this option will keep the display on the retardance image and not display raw images. This can be changed during series acquisitions

#### Processing:

*Enable On-the-fly Processing during Acquisition*: This option is available to disable processing for the computed images while acquiring. In the case where acquisition speed is extremely fast or the Image resolution is higher than usual and enough computation power is not available, to avoid any lag that could affect acquisition this selection can be used. This acquired dataset will then need to be Processed using the Pol-Analyzer interface. Default is enabled.

*Parallelize Processing:* This option will utilize all available CPU cores for processing the acquired images. Default is disabled.

#### Logger tab

Session Log Created: 2013.03.28 01:04:18 01:04:18 Session User: Rudolf Oldenbourg 01:04:18 Session Name: 2013_03_28_Birefringence 01:04:18 Mode: Birefringence 01:16:01 Acquired SMS_2013_0328_1314_1 : Image comment Session Exited: 2013.03.28 01:24:58
01:04:18 Session User: Rudolf Oldenbourg 01:04:18 Session Name: 2013_03_28_Birefringence 01:04:18 Mode: Birefringence 01:16:01 Acquired SMS_2013_0328_1314_1 : Image comment Session Exited: 2013.03.28 01:24:58
01:04:18 Session Name: 2013_03_28_Birefringence 01:04:18 Mode: Birefringence 01:16:01 Acquired SMS_2013_0328_1314_1 : Image comment Session Exited: 2013.03.28 01:24:58
01:04:18 Mode: Birefringence 01:16:01 Acquired SMS_2013_0328_1314_1 : Image comment Session Exited: 2013.03.28 01:24:58
01:16:01 Acquired SMS_2013_0328_1314_1 : Image comment Session Exited: 2013.03.28 01:24:58
Session Exited: 2013.03.28 01:24:58
 Section Records 2012 02 28 01:27:42
Session Resulted. 2015.05.28 01.57.42
Session Resumed: 2013.03.28 01:51:56
Session Exited: 2013.03.28 01:54:24
Session Resumed: 2013.03.28 01:54:53
Session Exited: 2013.03.28 01:55:53
Session Resumed: 2013.03.28 02:02:00
Session Exited: 2013.03.28 02:02:42
Session Resumed: 2013.03.28 02:04:50
Session Exited: 2013.03.28 02:05:46
Log Last Saved: 2013 04 11 04:41:28

This tab creates a chronological event log for the Session. The log file (session\_log.txt) resides in the User's session directory. Other than providing basic User information, all images acquired in Micro-Manager are logged in this log file. A User can also add their own comments and notes in the text area regarding their experiments.

**Note:** It is particularly useful for adding key changes to the system's hardware (eg. Objective, Filter wheel, etc.) that are not motorized and connected to Micro-Manager and thus their information is not logged in the image metadata.

Acquisition Buttons

cquire Birefringence		SM: Acquire Sample images
		BG: Acquire Background
SM BG	Series AutoClear	Series: Acquire Series/Multi Dimensional AutoClear: Erases the Acquisition
Birefringence: Preferences Saved		Comment after each acquisition.
cquire Birefringence		Stop: During Acquisition the Series
SM BG	Stop !	button functions as a Stop button.
Acquisition Comments		
Image comment	AutoClear	
Frames: 1/3 Channels: 7/7 End Time: 13:16 21:	5	

(These acquisition buttons are added to the TopFrame, if it has been started.)

**Note:** During Series acquisition, the display of the progress bar changes with each acquired image. However, the End Time is calculated at the start of series acquisitions and remains fixed. If the user pauses the series acquisition, the displayed End Time will not change and is therefore incorrect.

The Series/Multi-D Acq. uses Micro-Manager's built in Multi-D Acq. engine and dialog.

## Pol-Acquisition User Manual, Birefringence Imaging

Time	points		1	Acquisitio	n order —			Close
Number	r <u>3</u> -	*	-	Time, Slice,	Channel		•	
Interval	20		J				-	Acquire!
merver				Autofo	cus			Stop
Multi	ple position	s (XY) —			P Options	iii.		Level
[	Edit posi	tion list	12 II.	Skip fr	ame(s):	0 _		Load
	Eur posi					Ţ		Save as
Z-sta	cks (slices)	6		Summary				
7-sta	rt [um] 0	-		Number of ti	me points: 3			Advanced
7	d fuml			Number of p Number of s	lices: 2			
Z-end			et	Number of c	hannels: 5			
Z-ste	p [um] 2	_		Total images	: 30 v: 10 MB			
relat	tive Z	-		Duration: 0h	1m 0s			
				Orden Time	Cline Chan			
	Keep sh	utter open		order. rime,	Silce, chan	liei		
	Keep sh	utter open		order. Time,	Silce, Chan	liei		
Chan	Keep sh	utter open		order. Time,	Silce, chan	nei		
Chan	Meep sh	utter open el group: V	ariLC_PolA	lorder. Time,		] Keep shu	utter ope	en
Chan	Chann Config	utter open el group: V Exposure	/ariLC_PoIA Z-offset	Z-stack	Skip Fr.	] Keep shu Color	utter ope	en New
Use?	Config VariLC	el group: V Exposure 75	/ariLC_PoIA Z-offset 0	Z-stack	Skip Fr.	Keep shu Color	utter ope	n New Remove
Chan Use?	Chann Config VariLC VariLC	el group: V Exposure 75 75	/ariLC_PoIA Z-offset 0	Z-stack	Skip Fr.	Color	utter ope	n New Remove
V Chan	Channels Channels Config VariLC VariLC VariLC	el group: V Exposure 75 75 75 75	/ariLC_PolA Z-offset 0 0	Z-stack	<ul> <li>Skip Fr.</li> <li>0</li> <li>0</li> <li>0</li> </ul>	Color	utter ope	n New Remove
V Chan Use? V V	Channels Channels VariLC VariLC VariLC VariLC VariLC	el group: V Exposure 75 75 75 75 75	/ariLC_PolA Z-offset 0 0 0	Z-stack	<ul> <li>Skip Fr.</li> <li>0</li> <li>0</li> <li>0</li> <li>0</li> </ul>	Color	utter ope	Remove
Chan	Config VariLC VariLC VariLC VariLC VariLC images	el group: V Exposure 75 75 75 75 75	/ariLC_PolA Z-offset 0 0 0	Z-stack	<ul> <li>Skip Fr.</li> <li>0</li> <li>0</li> <li>0</li> </ul>	] Keep shu Color	utter ope	n New Remove Up Down
Chan	Chann Config VariLC VariLC VariLC VariLC VariLC images	el group: V Exposure 75 75 75 75 75	/ariLC_PolA Z-offset 0 0 0	Z-stack	<ul> <li>Skip Fr.</li> <li>Skip Fr.</li> <li>0</li> <li>0</li> <li>0</li> <li>0</li> </ul>	Color	utter ope	n New Remove Up Down
Chan Use? V V V Save Directory	Chann Config VariLC VariLC VariLC VariLC VariLC images root C:\LC	el group: V Exposure 75 75 75 75 75 75 75	/ariLC_PoIA Z-offset 0 0 0 0	Veq Z-stack V V V	Skip Fr.         0           0         0           0         0           0         0           0         0	Color	utter ope	n Remove Up Down
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Chan Use?  Save Saving for	Config VariLC	el group: V Exposure 75 75 75 75 75 2012_0914 ) Single-inr	fariLC_PoIA Z-offset 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Veq Z-stack V V Didenbourg Didenbourg	Skip Fr.     D	Color		n New Remove Up Down
Chan	Config VariLC VariLC VariLC VariLC VariLC vari vari vari vari vari vari vari vari	el group: V Exposure 75 75 75 75 2012_0914 Single-im	fariLC_PoIA Z-offset 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Veq Z-stack V V Didenbourg Didenbourg	Skip Fr.     D	Color		n Remove Up Down
Chan	Config VariLC VariLC VariLC VariLC VariLC VariC VariLC -	el group: V Exposure 75 75 75 75 75 2012_0914 Single-im nts	fariLC_PoIA Z-offset 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Voq Z-stack V V DidenbourgV	Skip Fr. 0 0 0 0 0 0 0 0 0 0 0 0 0	Color		n Remove Up Down
Chan Use? V V Save Directory Name pre Saving fo	Config VariLC VariLC VariLC VariLC variLC -	el group: V Exposure 75 75 75 75 75 2012_0914 Single-im nts	/ariLC_PoIA Z-offset 0 0 0 0 ata\Rudolf C	Voq Z-stack V V DidenbourgV	Skip Fr. 0 0 0 0 0 0 0 0 0 0 0 0 0	Keep shu Color		n Remove Up Down

**Note**: The *Channels* settings should not be changed at this stage.

**Note**: The Saving format that is used by the PolScope plugin is 'Single-image files' and <u>should NOT</u> be changed.

#### **Pol-Acquisition User Manual, Birefringence Imaging**



PolScope image stack: The information at the bottom of a PolScope stack includes pixel values in terms of the properties defined by the processor. The Birefringence processor will display the Retardance value of a pixel.

Orient. Lines: This check box causes an overlay of orientation lines to be drawn inside a specified ROI. Parameters such as the line interval are set with the Orientation-LinesV3 plugin button on the Viewer. In a series, the lines are recalculated for each time-point and/or z-slice.

The Pol-Acquisition routine writes metadata and comments in the Micro-Manager Metadata panel.

~ Background	No Background
~ Processed Using	5-Frame
~ Retardance Ceiling (nm)	8

Per-image comments	-image commen	its:
--------------------	---------------	------

The pixel value in the Image represents the Orientation in degrees.

### Help

## **Report Tool**

	10:	polscope@mbl.edu, averma@mbl.edu, gharris@mbl.edu, rudolfo@mbl.ed
Send	From:	Email address used for replyin
	Subject:	PolScope plugin Report
essage		

The Report tool can be accessed via Help in the Menu bar to send bug reports or suggestions.

## ChangeLog

Pol-Acquisition (MicroMan	ager)		X
	ChangeLog		
Installed Version:	2.02b (04-11-2013)		
Latest Version Available:	2.02b (04-11-2013)		
ChangeLog LCPolScope			
Date: Wednesday, April 10, 20	13 9:44:57 PM		
Message:			
converter for imageJ pol-stack	to MMgr dataset with additional o	ptions	=
Date: Tuesday, April 09, 2013 (	8:44:22 PM		
Message:			
converter for imageJ pol-stack	to MMgr dataset using Storage-A	pi	
(make-shift implementation to c	onvert Aster image) dPoiManager image window to ar	other	
dataset when Rois are defined	aromanager mage window to ar	lotifici	
added 'nm' Unit for Retardance	display images		
Date: Monday, April 08, 2013 9	:09:10 PM		
Added Birefringence and Dichr	oism User manual		
Added Birefringence and Dichr	oism User manual to installer		
Date: Monday, April 08, 2013 5	:10:39 PM		
Message: refactoring PoLAnalyzer			
relacioning FOFAnaly26			Ψ.

#### About



## Appendix: A

## Multi-Mode Acquisition

The following steps demonstrate how to setup Micro-Manager to acquire data using other modalities (Brightfield, Darkfield, Fluorescence, etc.) along with PolScope data using the OpenPolScope Pol-Acquisition software.

This guide assumes the necessary hardware has been configured using the Hardware configuration wizard.

**Step 1**: Start by creating a new Group using the + button and then naming eg. "Multi-D Group" and selecting all hardware that require to be changed between acquiring PolScope dataset and other type of datasets.

Configuration settings		Save
Group	Preset	
System	Startup	
Group: + -	Edit Preset: + -	- Edit

**Step 2**: In this example we change the Nikon TI Filter Block. In our setup the TIFilterBlock1-Label 1, TIFilterBlock1-State 0 houses the PolScope Analyzer.

Here you can specify the properties incluin a configuration group	uded Sho	W	ОК
-		cameras	Cancel
Show read-only properties	<b>M</b>	shutters	
	<b>⊻</b> :	stages	
		wheels, turrets, etc.	
Group name: Multi-D Group		other devices	
Property Name	Use in Group?	Current Prope	erty Value
TIFilterBlock1-Label		1	
TIFilterBlock1-Label TIFilterBlock1-State	V V	1 D	

**Step 3**: We then proceed to define a Preset using Filter Block Label 2 or State 1 not 'Label 1' which is being used for PolScope.

Here you can specify the property values in a configuration preset		ОК	
in a configuration preset.	_	Cancel	
Preset name: Acq-Preset1			
Property Name	Preset Value		
TIFilterBlock1-Label	22		
TIFilterBlock1-State	1		

**Step 4**: The defined "Multi-D Group" will now show up.

File Tools Plugins Help

···· ··· ····					
🛐 Snap	Camera settings		Configuration settin	gs	Save
🕅 Live	Exposure [ms]	12.0245	Group	Preset	
Album	Binning	1×1 🔻	Multi-D Group	Acq-Preset1	
			System	Placktu shal-2 2	
Multi-D Acq.	Shutter	<b>•</b>	TIFilte	erBlock1:State=1	
i 😪 Refresh	Auto shutter 🔽	Close			
Please <u>cite Micro-M</u>	<mark>anager</mark> so funding w	ill continue!			
ROI Zoo	m Profile A	utofocus			
		2	Group: + -	Edit Preset: +	- Edit
Image info (from camera): 2048 × 2048 × 2, Intensity range: 16 bits, 0nm/pix, Z=4910.80um					

**Step 5**: If there are more than 1 then proceed to add a second hardware state using the + button for Preset selecting 'Label 3'

Here you can specifiy the property values in a configuration preset		ОК
Preset name: Acq-Preset2	-	Cancel
Property Name	Preset Value	
TIFilterBlock1-Label	33	
TIFilterBlock1-State	2	

**Step 6**: We now have defined 2 Presets for acquiring non-PolScope images. File Tools Plugins Help

🛐 Snap	Camera settings	\$	Configuration settin	gs	Save
🕅 Live	Exposure [ms]	12.0245	Group	Preset	
	Binning	1×1	Multi-D Group	Acq-Preset1	
Album			System	Acq-Preset1	n
📘 Multi-D Acq.	Shutter	<b>•</b>		Acq-Preset2	
🤣 Refresh	Auto shutter 🔽	Close			
Please <u>cite Micro-N</u>	<u>lanager</u> so funding w	/ill continue!			
ROI Zoo	om Profile A	Autofocus			
		2	Group: + -	Edit Preset: +	- Edit
Image info (from ca	imera): 2048 X 2048 X	×2, Intensity rang	ge: 16 bits, Onm/pix, Z=4	1910.75um	



**Step 8**: Start Pol-Acquisition. Under Options in the Multi-Mode Acquisition panel enable both options.

Data VariLC Parameters Birefringence Processing Opti	ons
-Multi-Mode Acquisition	
Ask to merge with preconfigured Group	
Always ask for Pol related Hardware Settings on Meror	_

**Step 9**: Click on the "Series" button and the dialog below should pop-up. Select the group you want merged with PolScope acquisition if you have multiple Groups and hit OK.

<u> </u>	Configuration settings pre-exist. Would you like to merge them with PolScope for Acquisition?				
	Multi-D Group				
	OK Cancel				

**Step 10**: The dialog below should pop-up. The preset name will be populated and the only thing to be selected is the Label. Set it to 1 since 1 has the Analyzer cube. Hit OK.

Here you can specifiy the property values in a configuration preset	ОК	
Preset name: VariLC - State0 - Acquired Image		Cancel
Property Name	Preset Value	
VariLC-String send to VariLC	State0	
TIFilterBlock1-Label	1	
TIFilterBlock1-State	0	

**Step 11**: If 'Series...' button was clicked previously the Multi-D dialog will show up. At this point change the exposure if required for the Acq-Preset 1 and 2. Setup Time and Z if required. Proceed acquisition by clicking the "Acquire!" button.

If 'SM' button is used instead of 'Series...' the following dialog does not show up and a Single timepoint acquisition proceeds with the default settings.

🛛 🗖 Time p	ooints	Acquisi	ition orde	ır —			1	Close
Number		Channel				Ŧ		Acquire!
Interval	0 ms 💌	🗌 Auto	focus —				٦Г	Stop
- Dauttin	la nacitiona (100		<b>/</b> 0p	tions				
	ie posicions (XT)			_				Load
	Edit position list	Ski	p frame(s):		0 -		Save as	
- 🗔 Z otor	ka (aliaaa)	C						
- <u>-</u> 2-stat	KS (SICES)	Number of	if time poin	ts: 1				Advanc
Z-start	[um] -25 Set	Number c	f positions	: 1			1-	
Z-end	[um] 25 Set	Number of slices: 1						
Zestep (um) 2			it channels res: 7	× 7				
	taunt la	Total men	ory: 56 Mi	в				
relatio	re Z 🔽	Duration:	0h 0m 0s					
	🔽 Keep shutter open	Order: Ch	annel					
🛛 🗹 Chann	els							
	Channel group: VariLC_Pol	Acq	<b>T</b>	☑	Keeps	shutter o	pen	
Use?	Configuration	Expos	Z-offset	Z-s	S			New
	Acq-Preset1	12.0245	0	V	0		1	Remove
	Acq-Preset2		0		0		1-	
	VariLC - State0 - Acquired Image	12.0245	0		0			Up
	VariLC - State1 - Acquired Image	12.0245	0	V	0	-		Down
			1	_				

The final Acquired dataset should show a total of 9 channels (2 Acq-Presets + 7 Pol related)

If you are satisfied with how you have defined your hardware for the Acquisition you may disable the option 'Always ask for Pol related Hardware Settings on Merge'

Doing so will from now on only bring up Step 9 & 11 when using the 'Series...' button and only Step 9 when using the 'SM' button.

Data VariLC Parameters Birefringence Processing Options	
Multi-Mode Acquisition	
Ask to merge with preconfigured Group	
Always ask for Pol related Hardware Settings on Merge	

If any of the "Multi-D Group" related hardware is changed eg. Filter cube position then one will need to re-enable the above option once to redefine the position of the Analyzer.

## **Appendix: B**

# Calibrating the OpenPolScope for measurement of slow axis of birefringence using cheek cells

Microscope used for the following

**Step:1** After swabing cheek cells onto coverslip and immersing it in saliva (isotonic immersion), setup Kohler illumination. Cheek cells do not refract light much, so we need to have some type of contrast enhancement. Reducing the illumination aperture by stopping down the condenser NA (one of the simplest type of cohtrast enhancement ) works well. When Kohler illumination is setup and condenser NA is stopped down, we see something like this:



**Figure 1:** Kohler aligned microscope. The hexagonal aperture visible in the specimen plane is the field stop. The aperture stop of the condenser was closed down to be smallest.

**Step:2** Now open both the field stop and aperture stop slightly, choose a ROI over background region and calibrate the VariLC. Notice that as aperture stop is opened, image has better resolution, but poorer contrast. The results are explained in screenshots that follow:



**Figure 2a:** Calibration was done by choosing a ROI over background as shown. The above image was acquired with VariLC set to setting-1 after calibration.

Help		К,	
User: Shalin	Session: 2011_	01_30_OrientationVe	erificationCheekCell
Acquire SM	BG		Series
	Preferences s	aved.	
Data VariLC Para	ameters   Birefringence   Processing	Options	
Swing:	0.03 fraction	Reset LC	
Wavelength:	546.0 nm	Exercise LC	
Current	LC-A LC-B	<b>Intensity</b> 22654.798	
Defaults	Calibrate	Check	
Setting 0	0.238	7078.487	
Setting 1	0.268 0.481	23068.726	
Setting 2	0.238 0.51 -	23496.991	
Setting 3	0.238 🛫 0.45 🛫	22588.260	
Setting 4	0.209 0.481	22654.798	
	Extinction Ratio	253.35	

**Figure 2b:** At the end of calibration, the VariLC panel is populated with appropriate retardance values for LC-A and LC-B as shown above. High extinction ratio indicates that calibration was successfull.

**Step 3:** Acquire background. We notice in above image that there is uneven illumination of the specimen. This and similar other imperfections in the optics are accounted for by a background Polstack. Move to an area of coverslip, where no specimen is present and acquire a background stack by pressing "BG" button on Pol-Acquisition plugin.



Figure 3: Background captures effect of uneven illumination. The field-stop may be considered an uneven illumination at specimen plane.

**Step 4**: Acquire sample stack and ensure that the slow axis orientation is along the edge of the cell.



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