Pol-Analyzer, *OpenPolScope* software (ver. 3.0) for ImageJ

Last Updated: March 22, 2017; Revision 1.08

This manual was prepared with Pol-Analyzer software version 3.0 and Birefringence Processing enabled. Separate manuals are available for Dichroism and Fluorescence Polarization. Please refer under the Help section for those.

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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. The *Demo* version only installs the Pol-Analyzer plugin.

Software requirements

1. Micro-Manager (tested with release 1.4.22 subsequent changes in Micro-Manager code might result in unexpected behavior)
2. *OpenPolScope* software : https://openpolscope.org/pages/Downloads.htm
   Installer for Windows XP up to Windows10
   Installer for Mac

Note: Currently only VariLC and a Filter Wheel implementation is compatible with the Mac version for Acquisition.

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) dependant on camera.
5. 10 MB Hard Disk Space (additional space required for UserData)

Software:

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

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 convention — SM_2012_0904_0213_5

Prefix_Year_MonthDate_HourMin_Suffix

Prefix:
SM – Sample
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)*

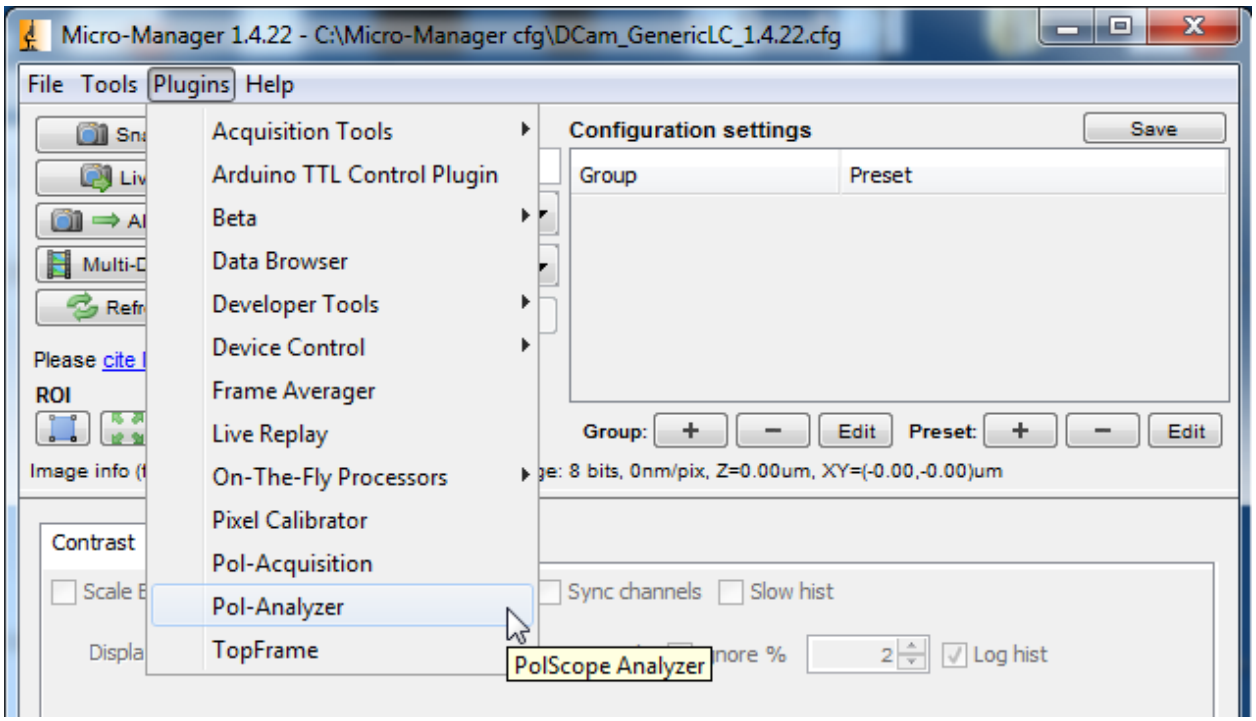
|

<SessionDate1> --- *(contains image and metadata files of a day's session)*

|

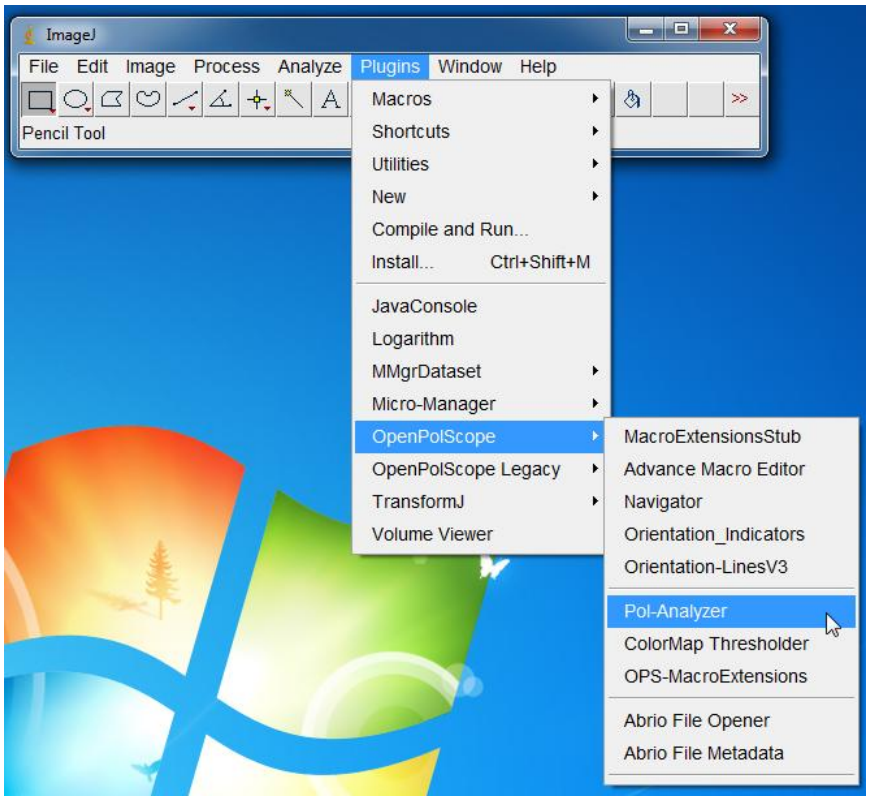
<SessionDate2>

Installation



For using the Pol-Analyzer plugin, a little familiarity with ImageJ is useful. The plugin is installed into the Micro-Manager plugins folder and appears under the Plugins menu, providing functions to view and analyze PolScope data that have been previously acquired. Image data need to be formatted as Micro-Manager stacks (not ImageJ stacks). Therefore, in its current release, Pol-Analyzer requires the co-installation of Micro-Manager.

When installed in Fiji or ImageJ The Pol-Analyzer plugin can be accessed from the ImageJ Plugins menu.



The OpenPolScope plugin suite for ImageJ contains a few extra useful plugins.

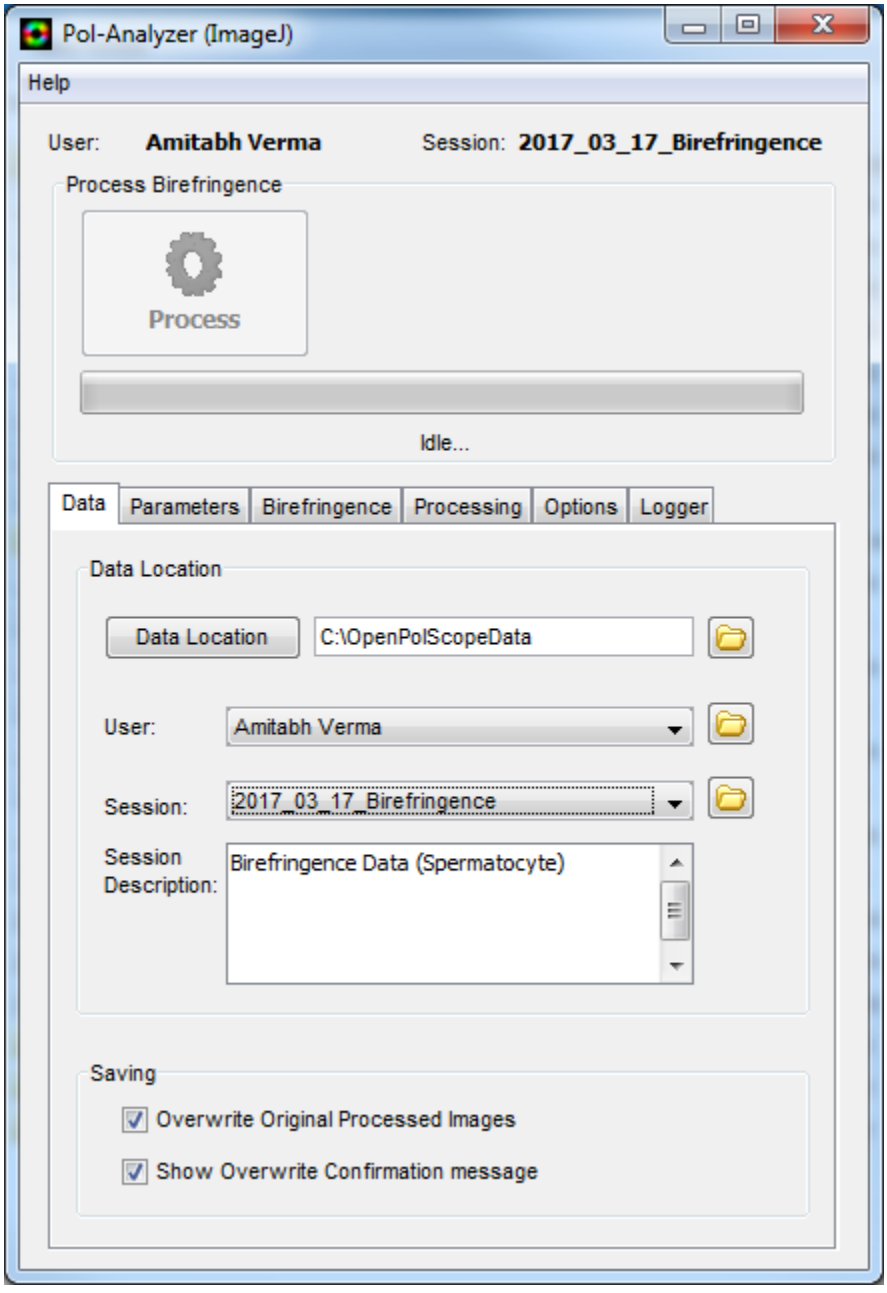
The Orientation-LinesV3 plugin can be used to set parameters in a single query panel and will operate on an open and active PolScope stack (Micro-Manager and ImageJ stack format).

The Abrio File Opener/Metadata supports opening Abrio acquired image datasets that typically have a file extension (*.pli).

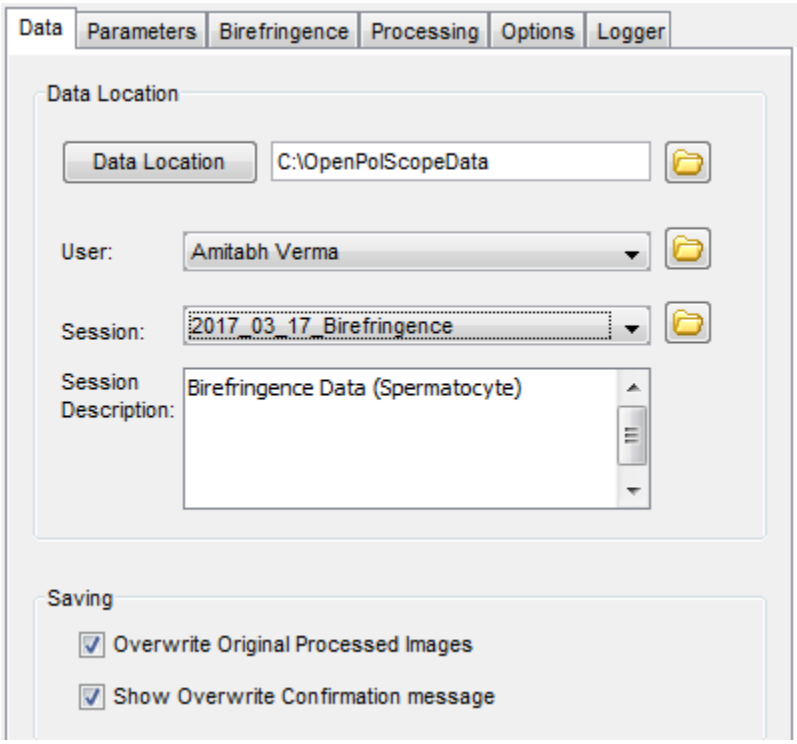
The PolScope Legacy entry contains plugins that operate only on traditional ImageJ PolScope stacks (ImageJ stacks)

Pol-Analyzer Window

The Pol-Analyzer window contains action buttons, a progress bar, and several tabs that let the user set data locations, measurement parameters, options, etc. The following sections give an overview of the window components.



Overview of Functionality and Tabs



The top line shows the folder names of the current User/Project and Session.

The Process button initiates the recalculation of the image data as specified by the settings in the tab panels

The progress bar gives an estimate of the completion of an active process.

Status messages are printed below the bar.

Data tab

In the Data tab panel, the root folder *Data Location* can be selected, where Micro-Manager data were saved. The lists of existing *User* and *Session* folders are then loaded into their corresponding pull down menus.

Note: The PolScope-Analyzer only works on existing data. Use the Pol-Acquisition plugin to acquire new PolScope images

Session Description: This description is imported when the Session was created and can be updated during Processing.

Saving

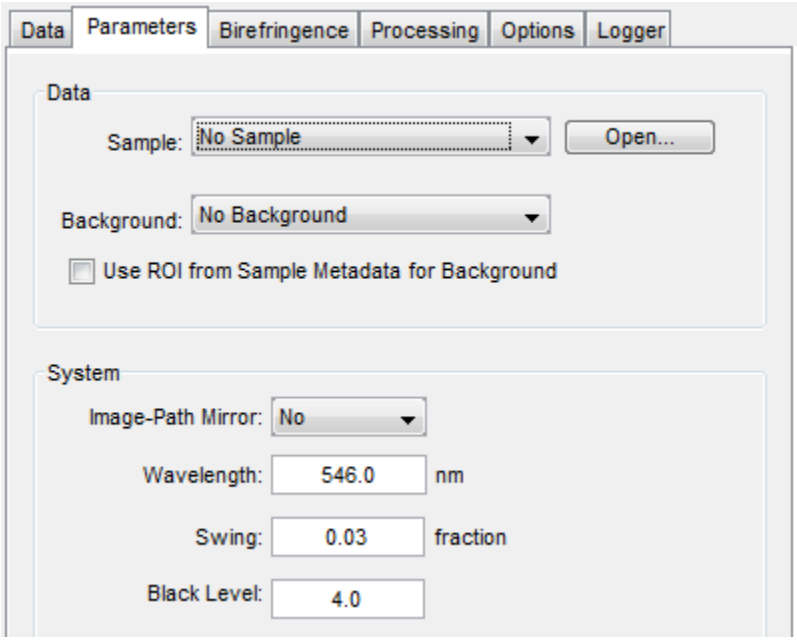
Overwrite Original Processed Images: Enable this option to overwrite previously computed images. Default is enabled.

Show Overwrite Confirmation message: When enabled, prompts for a confirmation before previously computed data are overwritten. Default is enabled.

Note: If overwrite is enabled, only processed images are overwritten. Raw images are never modified or overwritten.

Note: Newly computed data can only be saved by overwriting previous results at the time of generating the new results. If *Overwriting Original Processed Images* is not checked, the newly computed data, displayed in the stack, can only be saved to disk as a new, complete data stack, because Save commands in Micro-Manager don't allow overwriting previous data.

Parameters tab



This tab lets the user select the image window that holds the sample data (Processed Data) to be processed and the associated background data, if available. Pull-down menus let the user choose between available datasets. Depending on the check box settings in the Options panel, the entries in the sample pull down menu vary. The Open button lets the user open sample data from the disk.

The tab also shows measurement parameters at the time of acquisition or last processing. The information displayed here is stored in the metadata file and parsed when the dataset is loaded.

These parameters should not require any modifications. However, if background correction was not applied during acquisition or other parameters were not correct, the values can be edited and once reprocessed are saved to the image metadata.

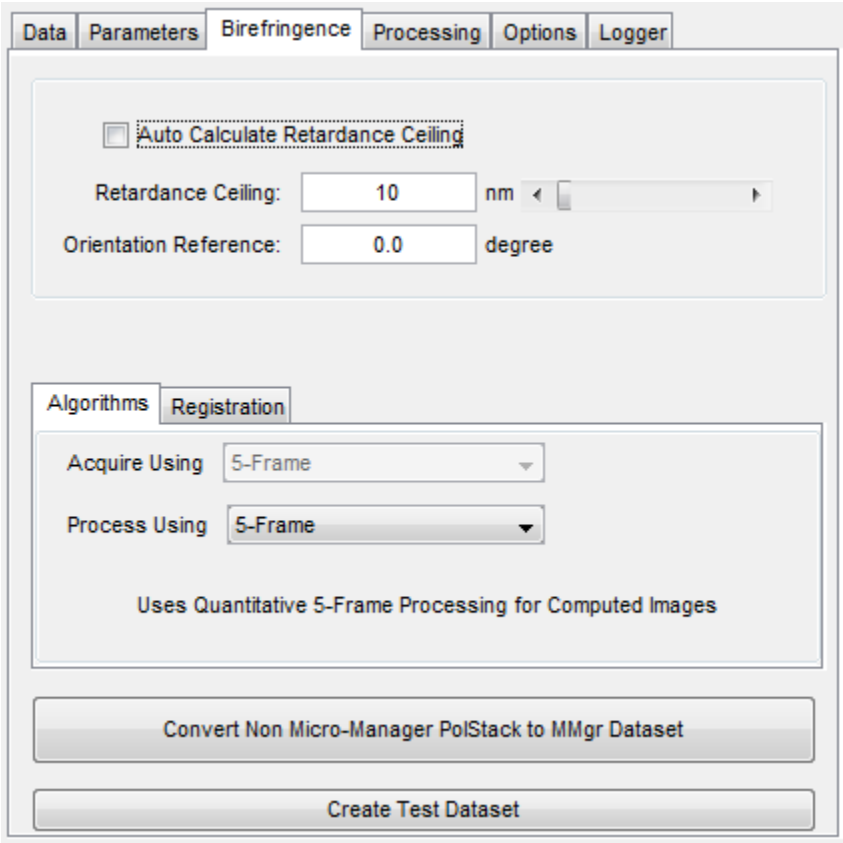
Image-Path Mirror: Used for an Inverted microscope or if there is a mirror in the optical path. Setting the mirror parameter inappropriately leads to incorrectly computed orientation values. Retardance values are not affected by the mirror setting.

Wavelength: Sets the wavelength that will be used when setting the retardance values. Default is 546 nm.

Swing: Sets the nominal retardance bias (as a fraction of the wavelength) for the elliptical settings of the liquid crystals.

Black Level: The blacklevel is the average pixel value that is measured when the camera is not illuminated. The black level primarily depends on the camera offset setting.

Birefringence tab



The main processor selected in the Processing panel (here Birefringence) will be displayed as an additional tab. Its panel will contain options only applicable when this processor is selected.

Retardance Ceiling: Sets the value of the retardance ceiling that is used for storing the retardance values in its image channel (usually first image). By way of explanation, all image data are stored as 8 bit or 16 bit pixel values, including the computed or virtual channels. For display and storage, computed retardance values are converted to 8- or 16-bit integer values, linearly mapping the retardance between 0 and the ceiling value into 0 and 255 (8-bit) or 0 and 4095 (16-bit). 4095 was chosen to make the converted retardance values similar to the intensity values acquired by a 12-bit camera. Retardance values that are computed to be higher than the ceiling value are stored as 255 or 4095.

Auto Calculate Retardance Ceiling: When enabled the software will calculate the retardance based on the highest retardance value it finds within the calculated retardance image. This might not be a true representation of the data in cases where there is debris within the sample space.

Orientation Reference: Sets the orientation reference that is used for processing the slow axis orientation image. (See Pol-Acquisition manual, sections “Calibration of calculated retardance and orientation values” and “Mapping of calculated retardance and orientation values into 8-bit and 16-bit images”)

Algorithms

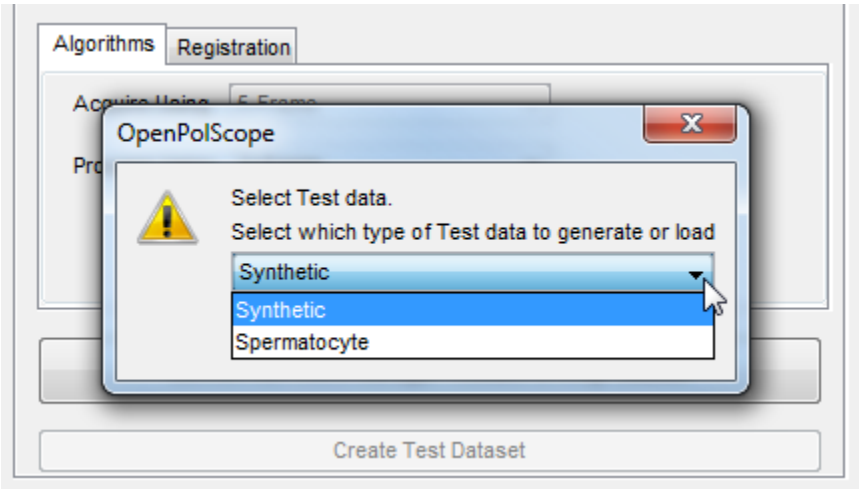
Acquire Using: Option to acquire only 4 frames instead of the default 5 frames, dropping the acquisition of the raw image for Setting 4.

Process Using: Option to use only 4 frames for processing instead of 5 frames.

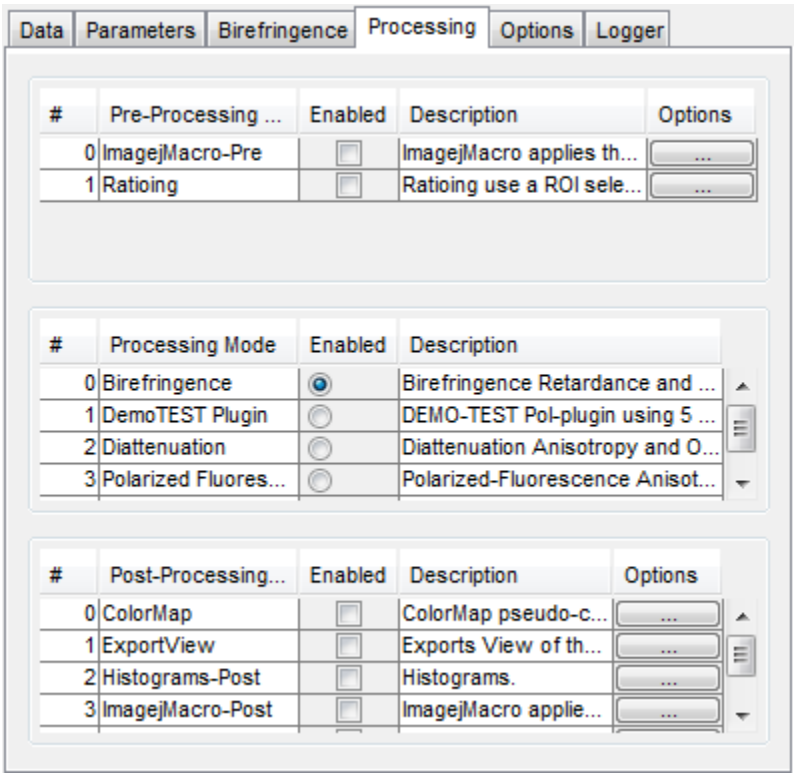
Note: 5-frame processing requires an activation key. This is available for free for all VariLC users and those Meadowlark LC users who have purchased the unit via the OpenPolScope Resource at MBL. Please send us a note to request an activation key.

Convert Non Micro-Manager PolStack to Mmgr Dataset : This option allows converting PolStack acquired using Psj or any software to be converted as a Micro-Manager PolStack. Default values might be used to populate metadata field and once converted the new dataset should be re-processed with correct parameters (eg. Background, retardance ceiling, swing, etc.)

Create Test Dataset: This button allows either loading of a test dataset or creating a test dataset based on User defined parameters. Additional test datasests can be downloaded from the OpenPolScope website https://openpolscope.org/pages/Sample_Datasets.htm



Processing tab



The Processing tab is divided into 3 main segments.

PreProcessing: 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 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 postprocessing.

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

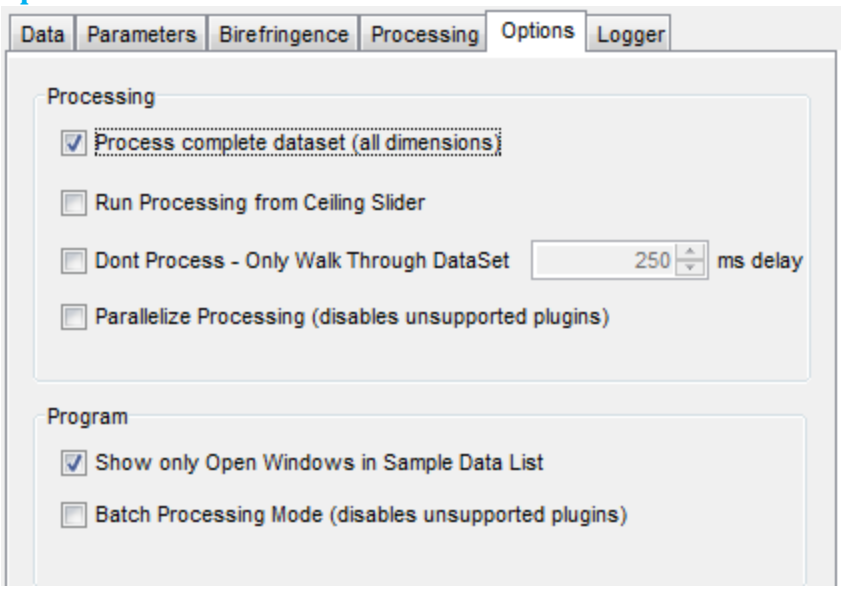
PostProcessing: This applies some post-processing correction to the images. It can also be used to generate additional images. Multiple *Postprocessors* can be selected.

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

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

Note: If a PolScope data set is opened and the associated *Processing Mode* plugin is not installed, the data-set will open as a regular Micro-Manager data-set and not a PolScope data-set.

Options tab



In the Options tab one can set preferences for the Pol-Analyzer.

Processing:

Process stacks: If checked, processing occurs for all computed channels of a data-set (stack); if not checked, processing occurs only for the currently selected time-point and/or z-position. Default is enabled.

Run Processing from Slider: When enabled will execute processing whenever a value is changed from the slider (eg. Retardance ceiling slider). Default is disabled.

Dont Process...: This option allows walking the data-set through time, slice, position without processing. The currently selected channel is always displayed.

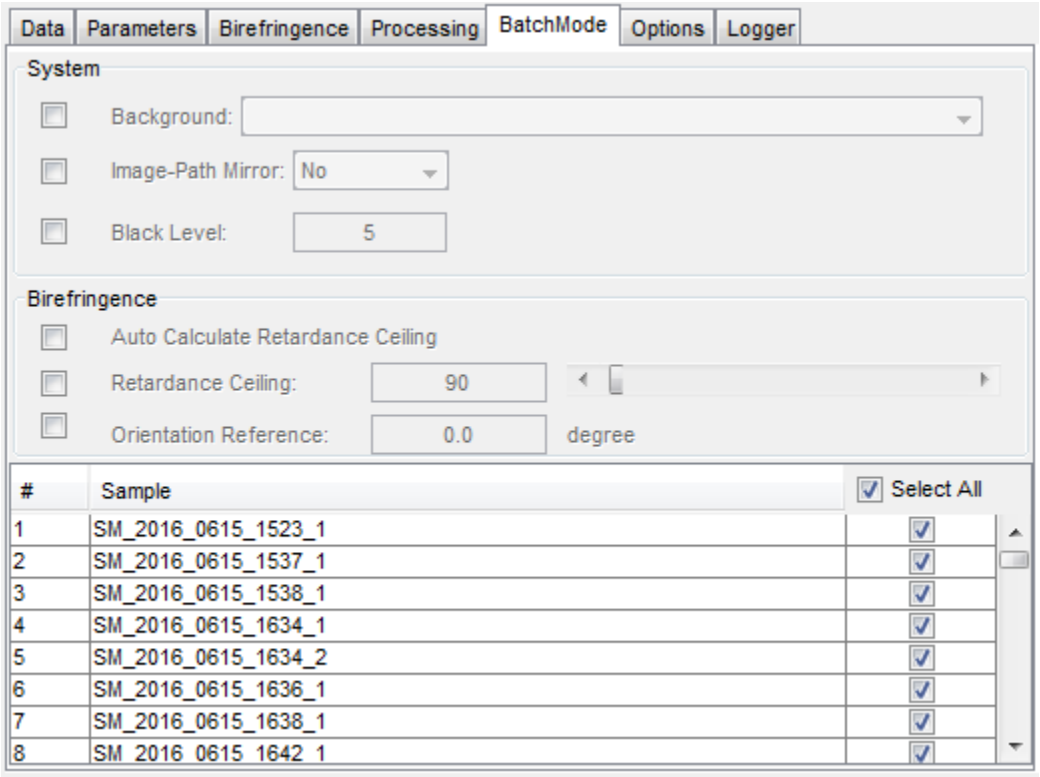
Parallelize Processing: Enables processing using multiple cpu cores that are available. This option provides full utilization of processing power when processing a Multi-dimentional stack. This should not be enabled when acquiring images and Processing at the same time. Default is enabled.

Program*:*

Show only Open Windows...: Modifies the behavior of the 'Open...' button under Parameters to List view instead of File dialog opener. Default is enabled.

Batch Processing Mode: Selecting this option provides a BatchMode panel for batch processing multi datasets. The Process button is transformed for 'Batch Process' and its corresponding panel is added next to Options panel.

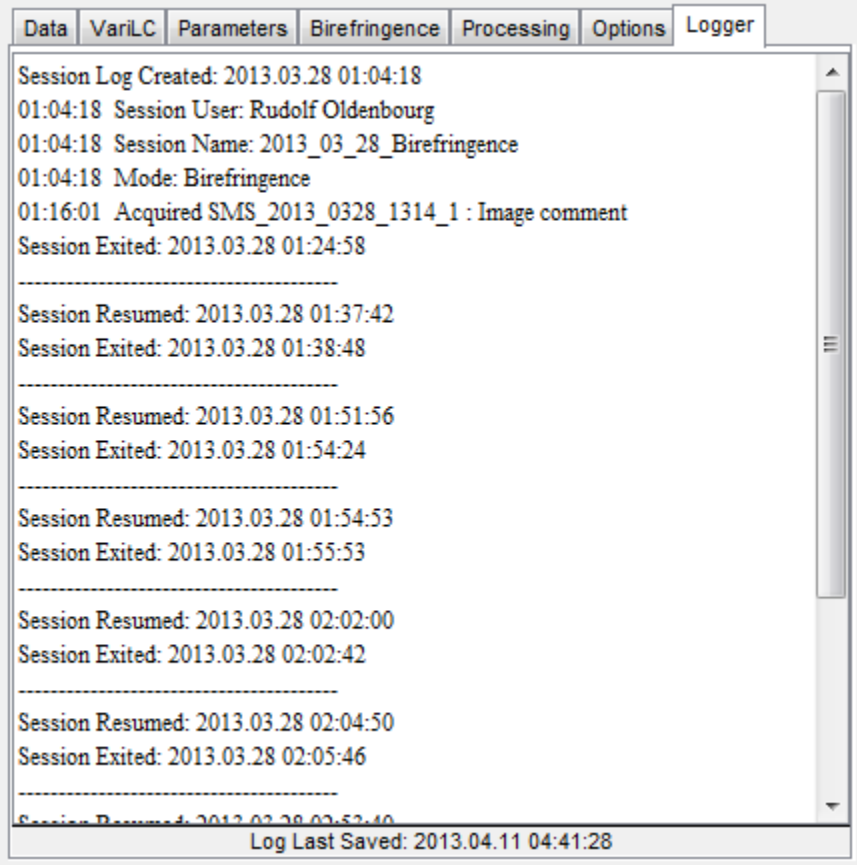
BatchMode tab



Batch Processing allows certain parameters that are Batch Processable to be applied to all datasets within a session. Once selected by enabling their checkbox, those values and/or selections will be applied to all the datasets that have been selected for Processing.

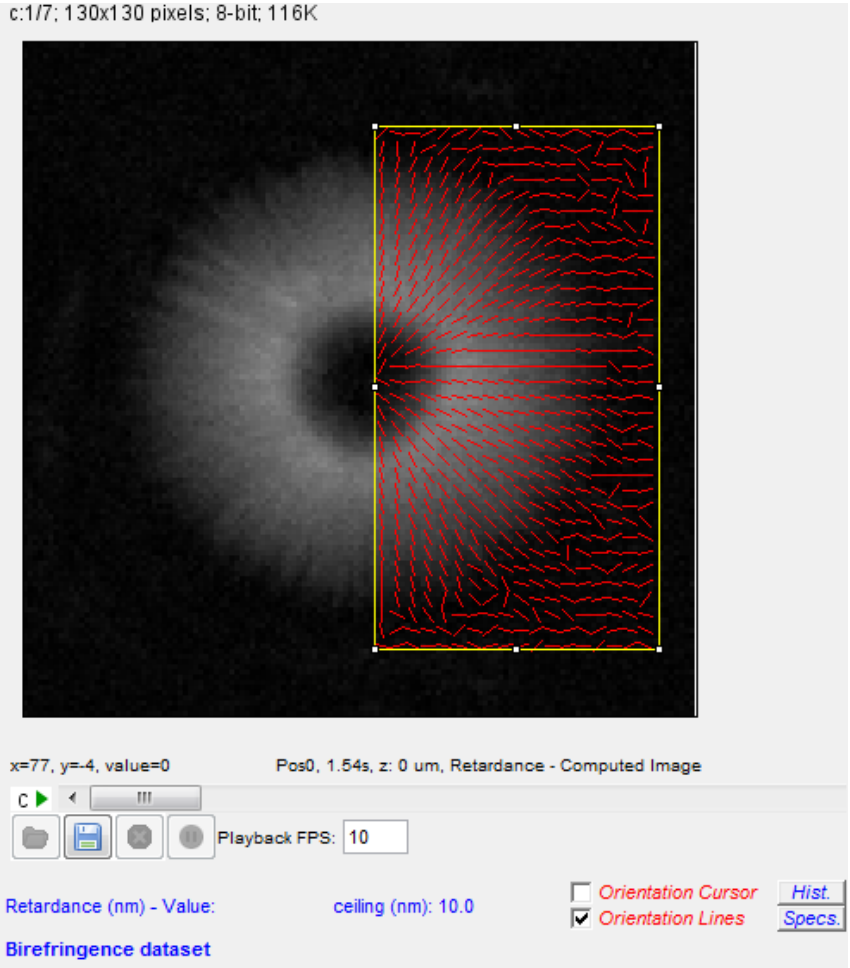
Note: During Batch Processing mode 'Open windows' will be ignored and the datasets required for processing can be selected from the panel's table-view. The currently selected Processing Mode is the default method used. If a Session consists of different type (different modes) of acquired data, those other datasets will be skipped when processing. Datasets that cannot be processed will continue to have their checkbox tick marks while the ones that have been processed will have their tick marks removed.

Logger tab



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.

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.



Orientation Cursor: This checkbox transforms the image cursor to a line cursor that displays the orientation of a pixel when hovered over the image.

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 re-calculated for each time-point and/or z-slice.

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

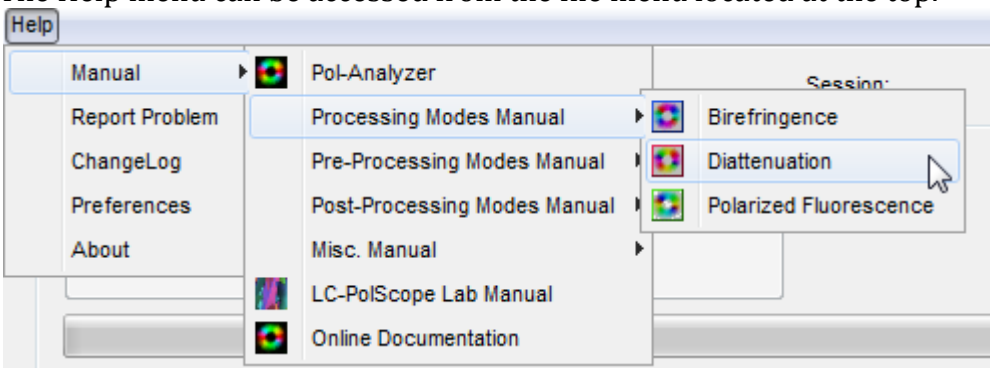
~ Background	No Background
~ Processed Using	5-Frame
~ Retardance Ceiling (nm)	8

Per-image comments:

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

Help

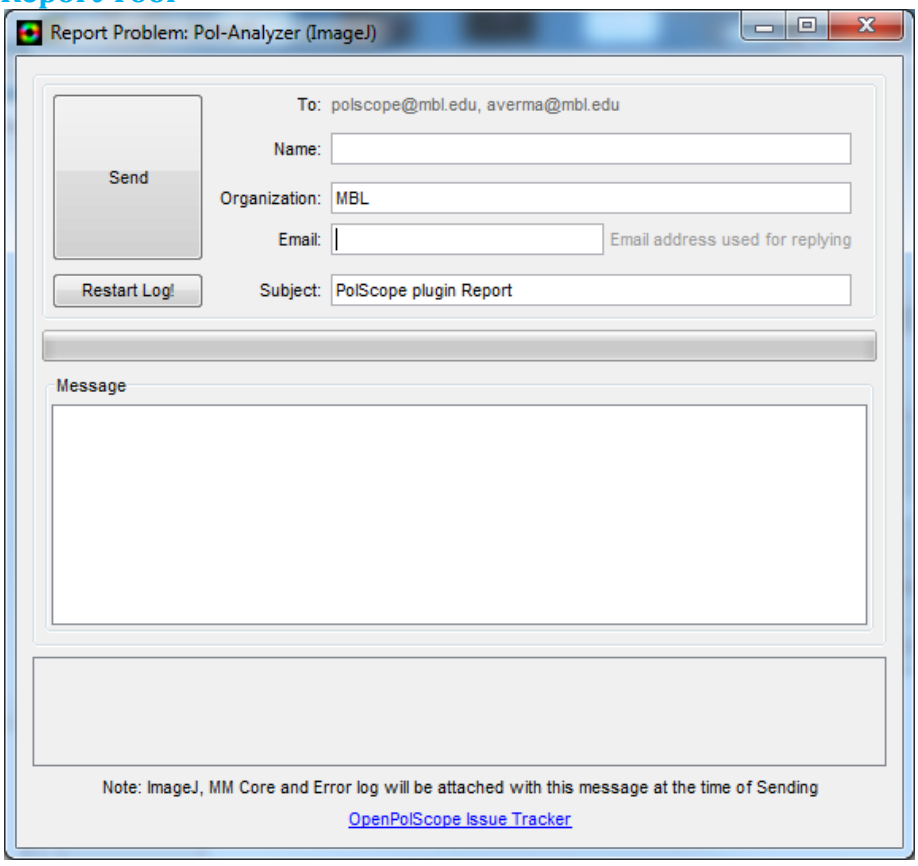
The Help menu can be accessed from the file menu located at the top.



Manuals

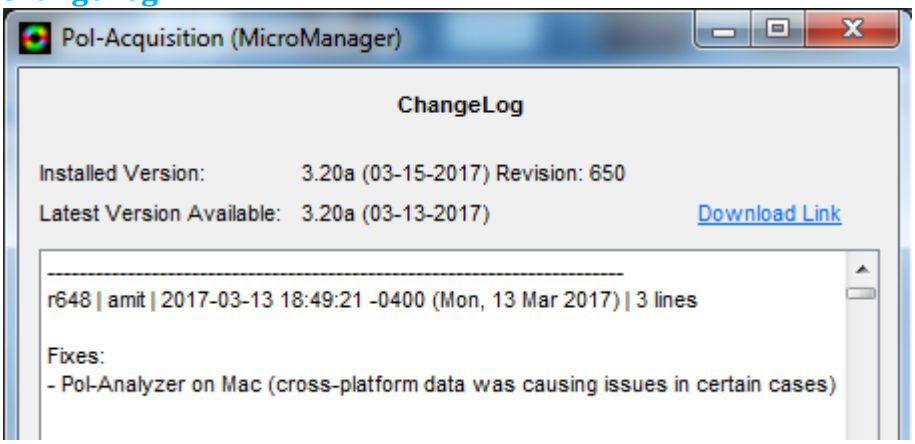
More helpful manuals, protocols and support files are available under this section including a link for online Documentation section of the OpenPolScope website (https://openpolscope.org/pages/Documentation.htm)

Report Tool



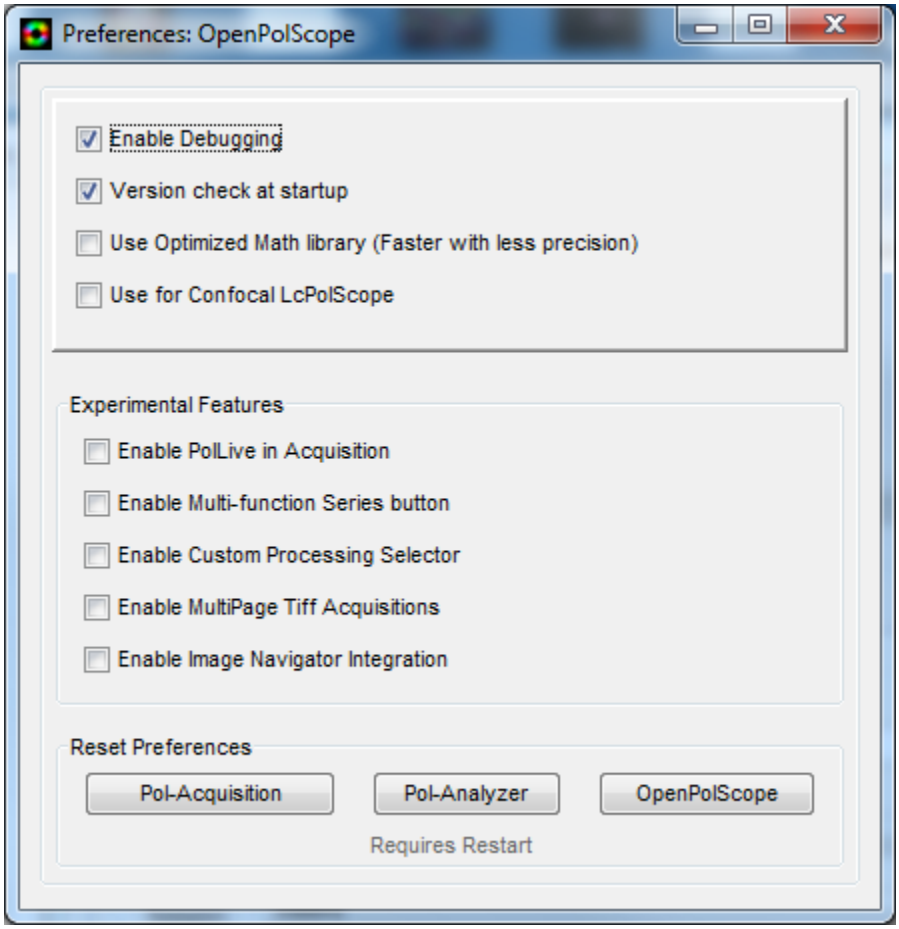
The Report tool can be accessed via Help in the Menu bar to send bug reports or suggestions.

ChangeLog



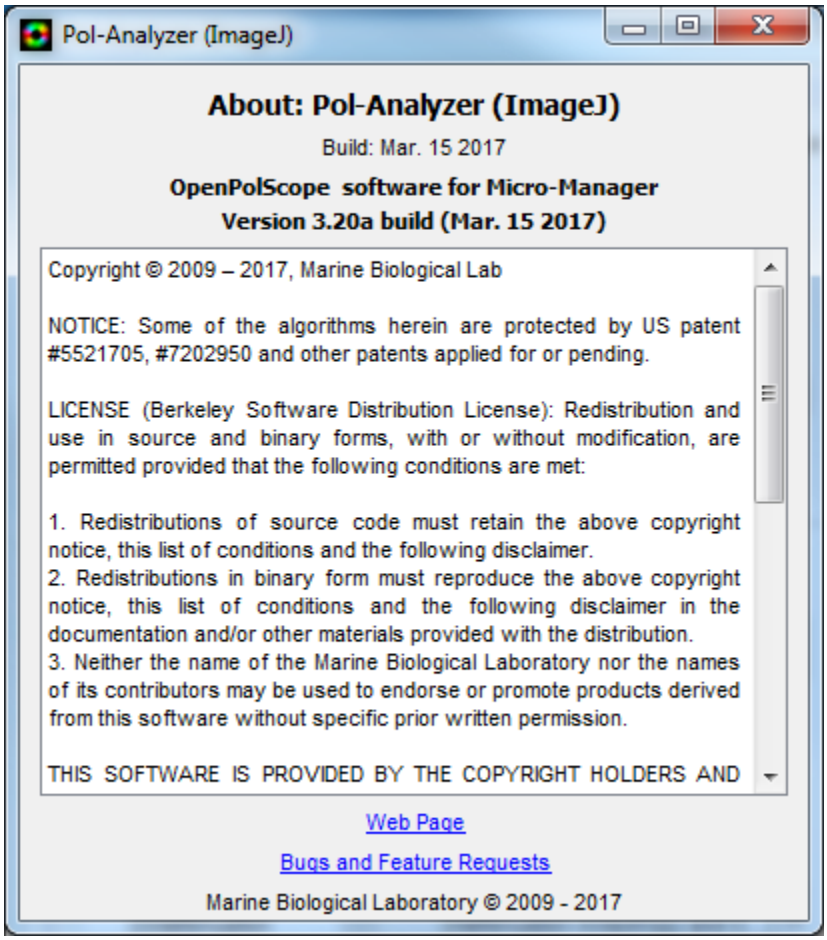
The ChangeLog allows viewing the latest changes and fixes that are incorporated in each new version released.

Preferences



The Preferences panel allows access to experimental options that are currently in test phase of development. They should only be enabled once advised by an OpenPolScope team member.

About


Pol-Analyzer (ImageJ)
About: Pol-Analyzer (ImageJ)
Build: Mar. 15 2017
OpenPolScope software for Micro-Manager
Version 3.20a build (Mar. 15 2017)
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Appendix: A

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

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:

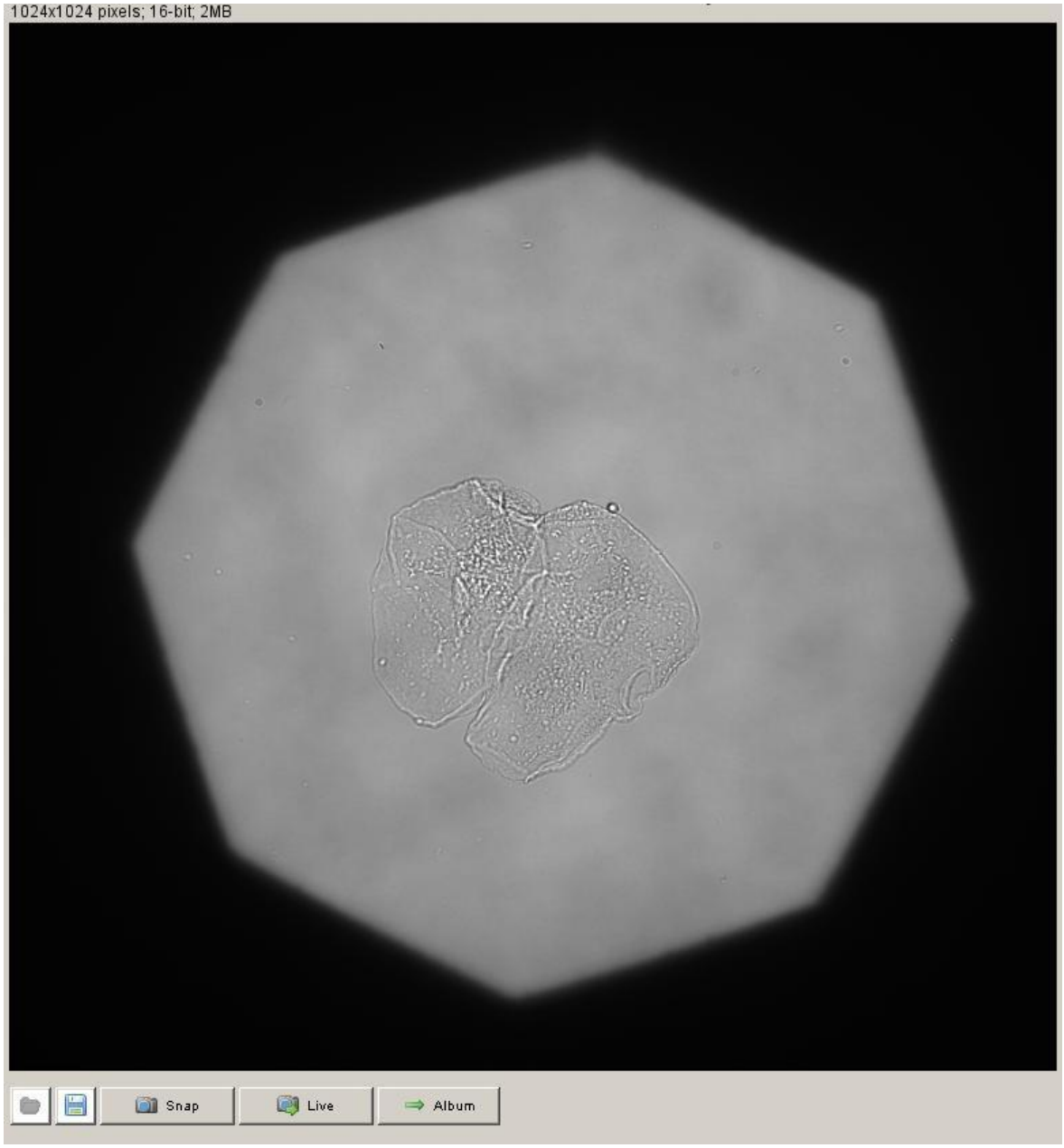


Figure 1: Kohler aligned microscope. The hexagonal aperture visible in the specimen plane is the field stop. The aperture stop of the condenser was closed down to be smallest.

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:

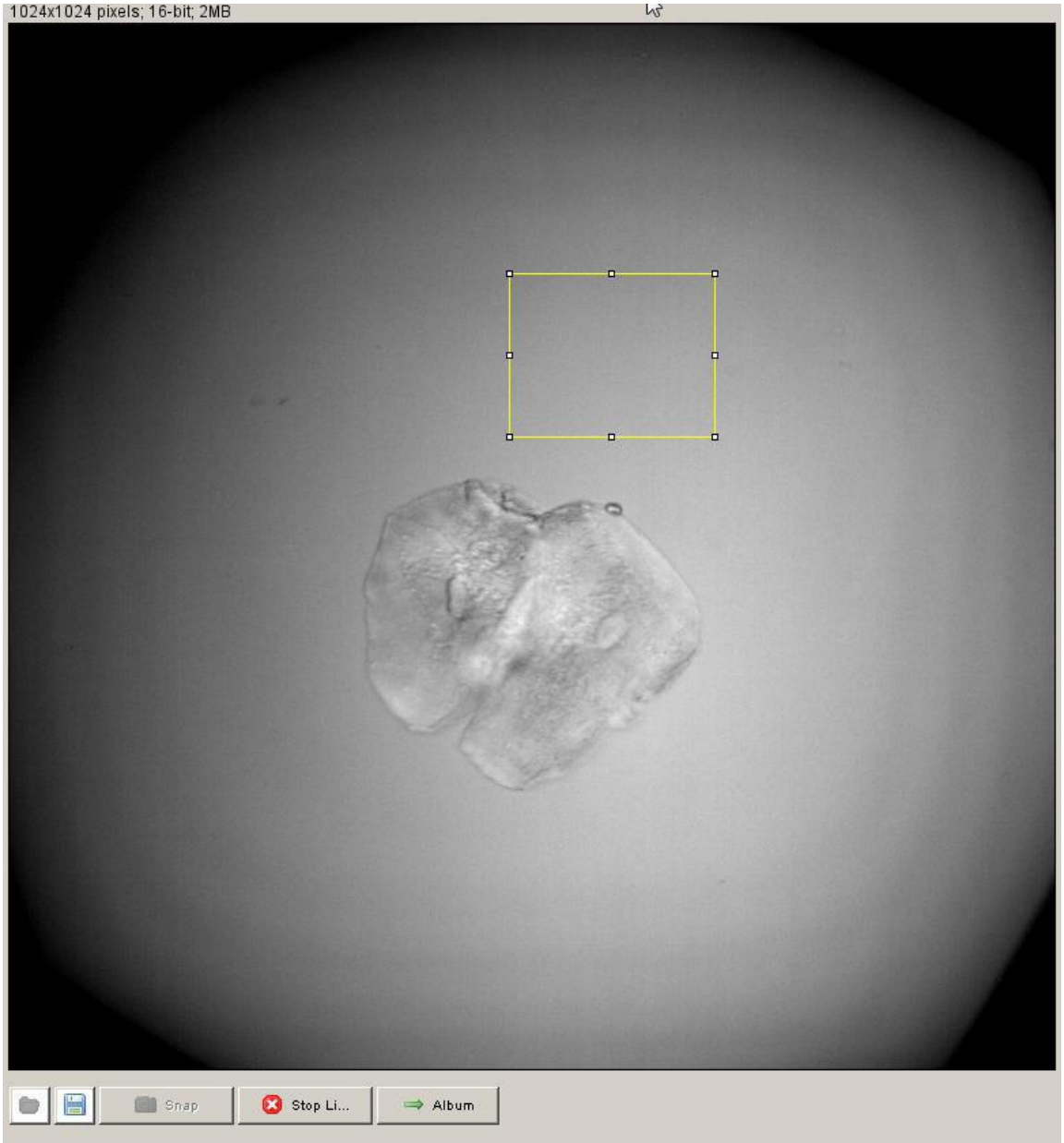


Figure 2a: Calibration was done by choosing a ROI over background as shown. The above image was acquired with VariLC set to setting-1 after calibration.

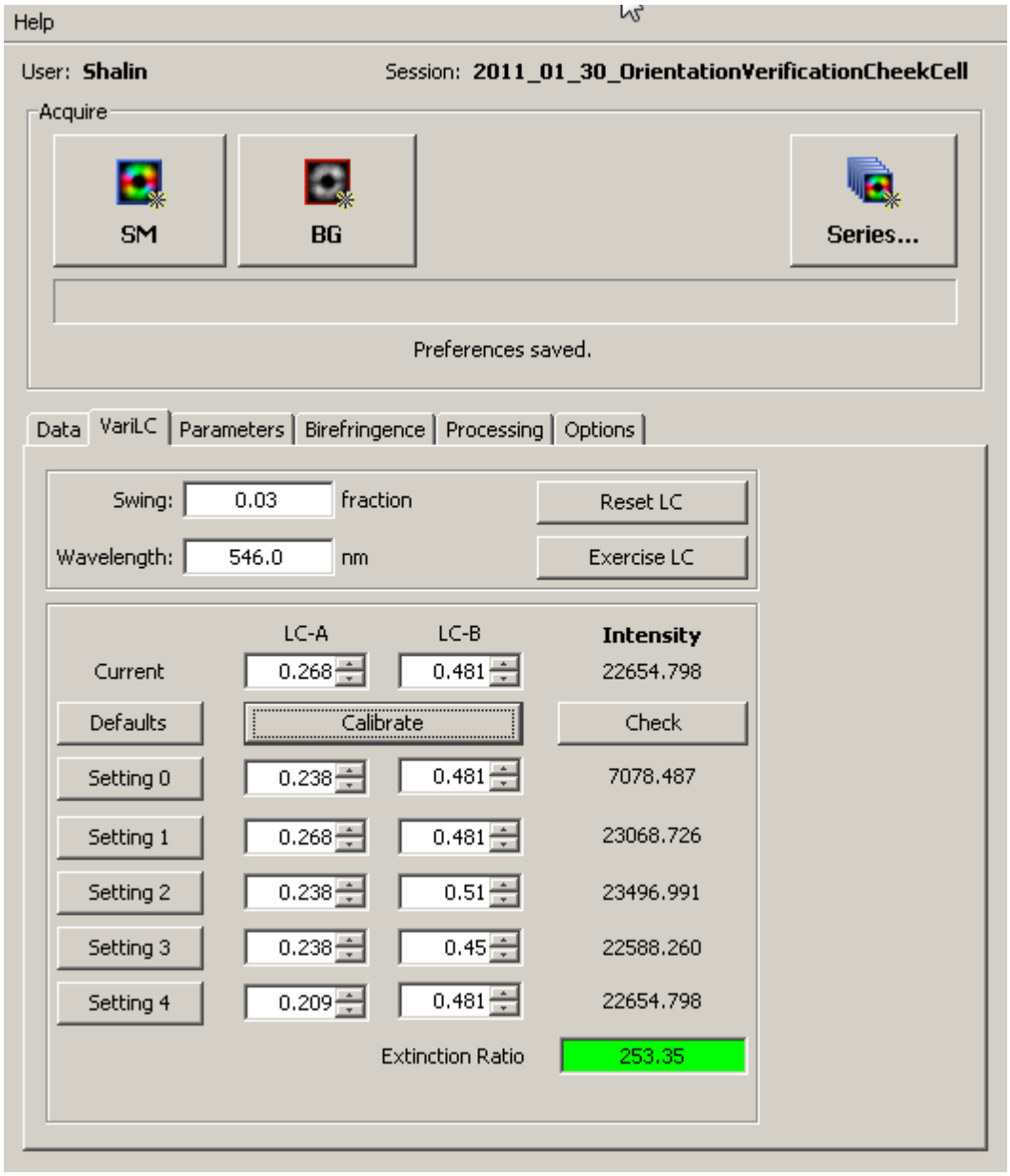


Figure 2b: At the end of calibration, the VariLC panel is populated with appropriate retardance values for LC-A and LC-B as shown above. High extinction ratio indicates that calibration was successful.

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.

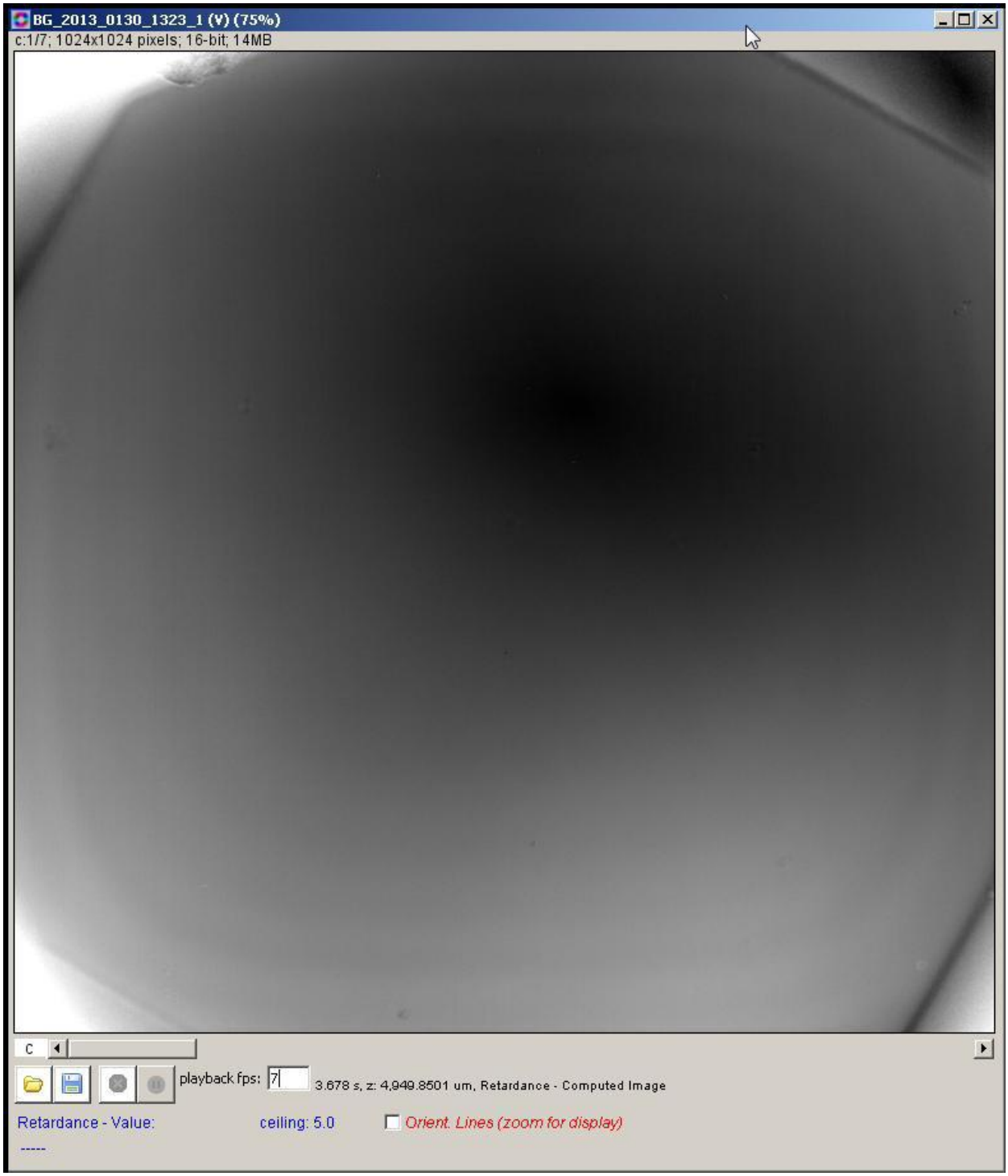
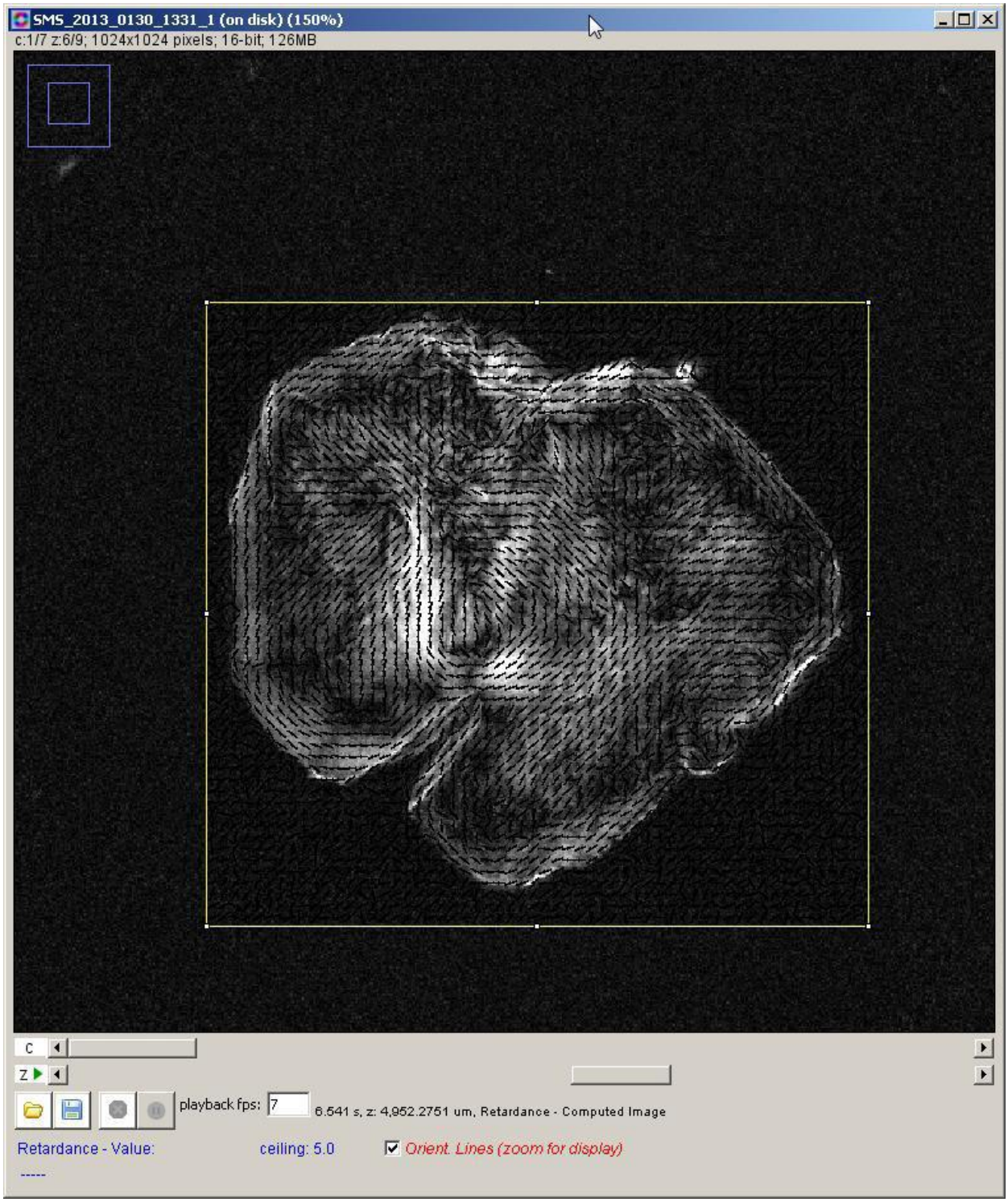


Figure 3: Background captures effect of uneven illumination. The field-stop may be considered an uneven illumination at specimen plane.

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



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