Pol-Analyzer, *OpenPolScope* software for ImageJ

Last Updated: April 15, 2013; Revision 1.07

This manual was prepared with Pol-Analyzer software version 2.0

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

Table of Contents

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; Birefringence; 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:**

1. 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 128MB or more if images are kept in RAM.

Guide to abbreviations

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

Image data is expected to be organized 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, the user needs to be familiar with ImageJ. The plugin is installed into the plugins folder and appears in the ImageJ 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.
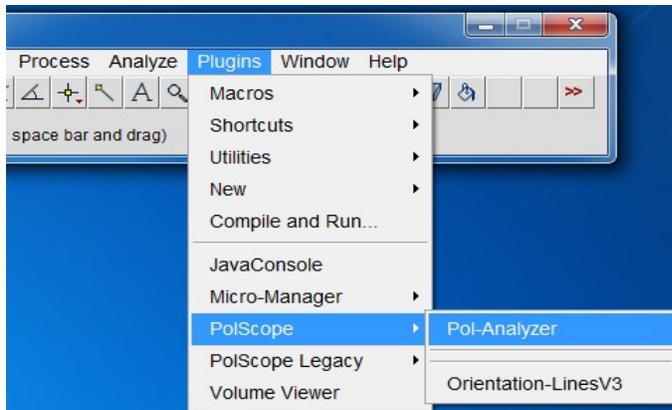
The Pol-Analyzer plugin can be accessed from the ImageJ Plugins menu.

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

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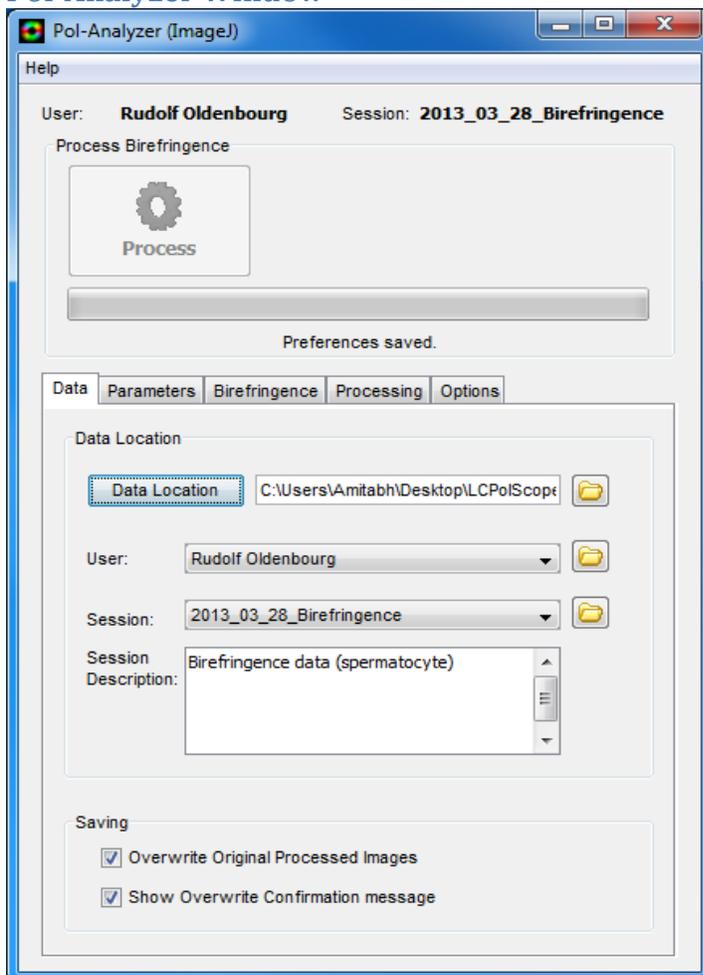
The Pol-Analyzer window contains action buttons, a progress bar, and several tabs that let the user set data locations, measurement parameters, options, etc. The following sections give an overview of the window components.

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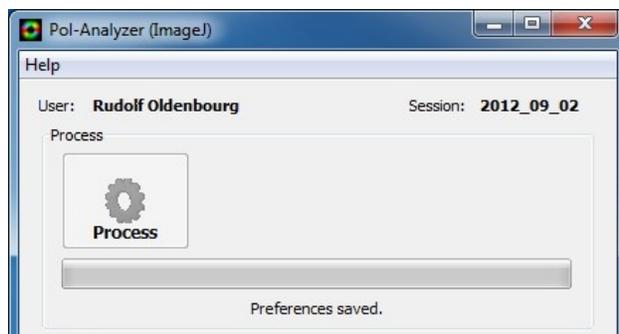
The top line shows the folder names of the current User/Project and Session.

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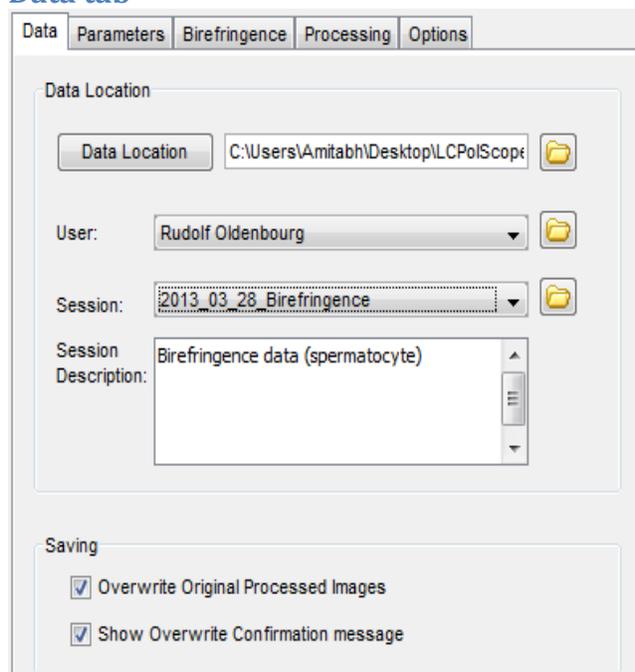
In the Data tab panel, the root folder *Data Location* can be selected, where Micro-Manager data were saved. The lists of existing *User* and *Session* folders are then loaded into their corresponding pull down menus.

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.

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")

Use 4-Frame Processing...: Option to use only 4 frames for processing, dropping the last raw image channel.

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.)

Processing tab
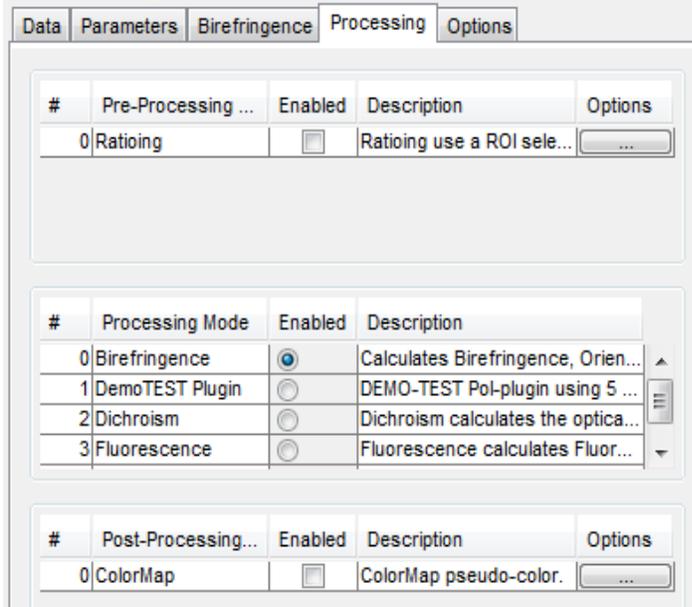
The Processing tab is divided into 3 main segments.

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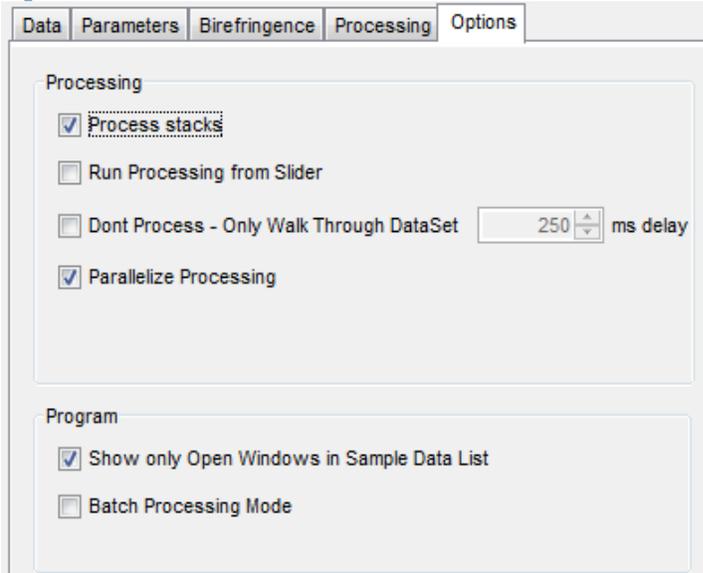
In the Options tab one can set preferences for the Pol-Analyzer.

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.
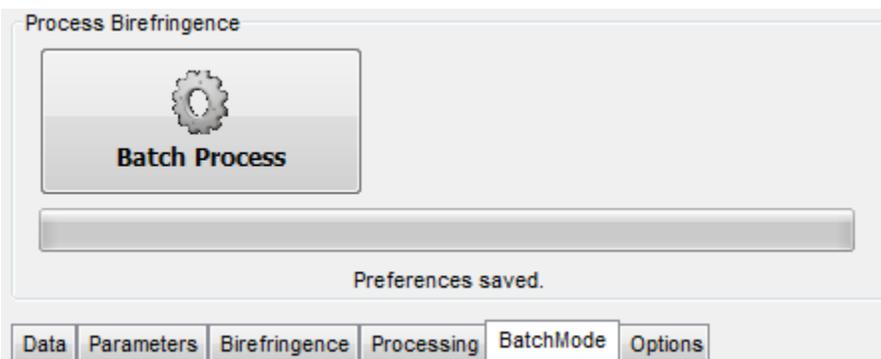
Note: During Batch Processing mode 'Open windows' will be ignored and the datasets required for processing can be selected from the panel's table-view. The currently selected

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.

BatchMode tab

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

Help

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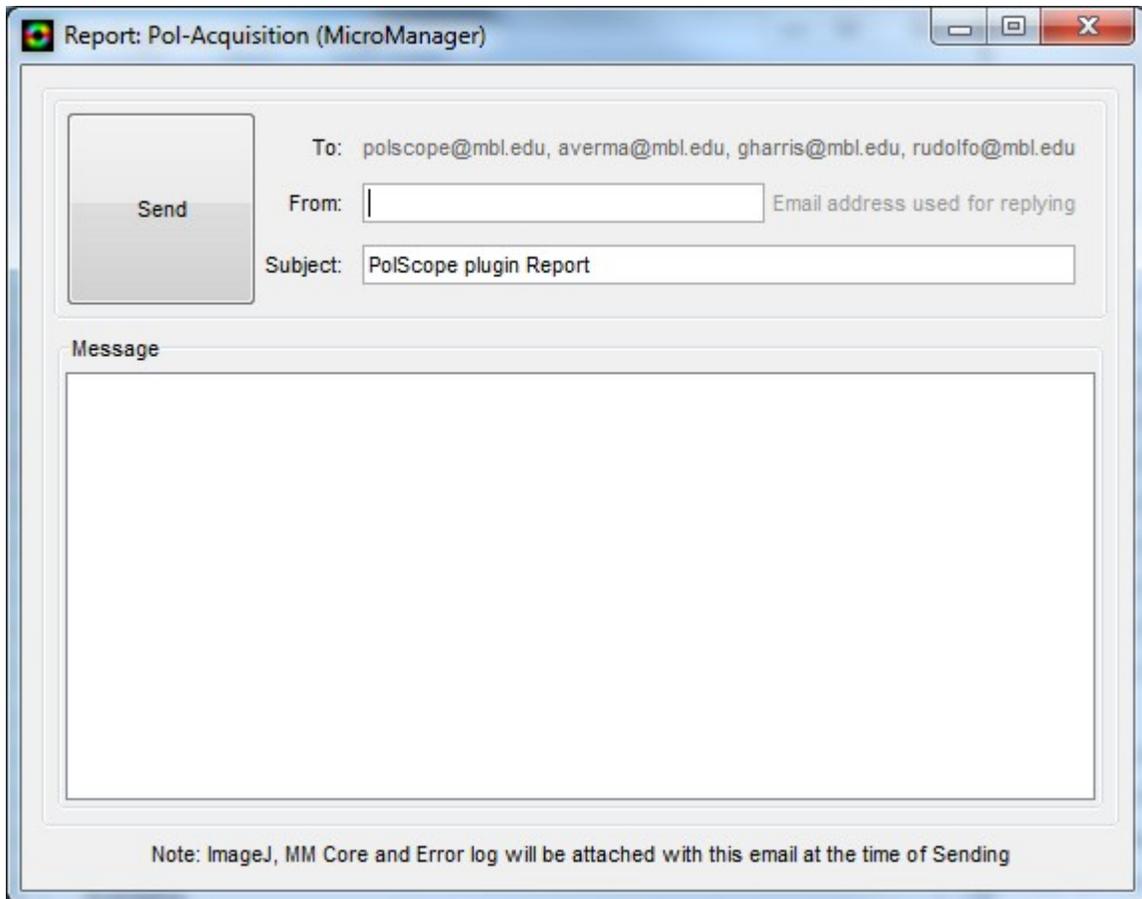
The Report tool can be accessed via the Menu bar to send bug reports or suggestions.

ChangeLog

Pol-Analyzer (ImageJ)

ChangeLog

Installed Version: 2.02b (04-15-2013)
Latest Version Available: 2.02b (04-15-2013)

----
r806 | amit | 2013-04-12 18:53:14 -0400 (Fri, 12 Apr 2013) | 2 lines

- Added option under Calibration for searching Maxima under Calibration Options
- Automated installer building and updating changelog info via Installer build script
----
r805 | amit | 2013-04-11 21:13:53 -0400 (Thu, 11 Apr 2013) | 5 lines

- fixed missing lib matlabcontrol used by PolScope API in installer script
- Changes to Tuning panel in VariLC - moved calibration options from Options and
renamed 'Tuning' panel to 'Calibration Options'
- Changed wordings for selections in Options
- Updated ChangeLog to use flexible (updatable) links for displaying content
- 1st draft of Pol-Acquisition manual
----
r804 | amit | 2013-04-10 21:44:57 -0400 (Wed, 10 Apr 2013) | 1 line

converter for imageJ pol-stack to MMgr dataset with additional options

About

Copyright, license, warranty/disclaimer

From the Laboratory of
Rudolf Oldenbourg (rudolfo@mbl.edu)
Cellular Dynamics Program
Marine Biological Lab,
Woods Hole, MA
USA
© 2013