Orientation Lines, OpenPolScope software for Micro-Manager

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Introduction

About

Orientation Lines is a visualization tool, part of the OpenPolScope Acquisition and Analysis software package. It works on PolScope datasets acquired using Pol-Acquisition or other acquisition tools that produce an Anisotropy, Orientation, Average (optional) images. It works both under Micro-Manager and as a standalone ImageJ plugin (using OpenPolScope-API and Micro-Manager as java libraries).

New features in this version:

  ➢ Fan glyphs : visualize anisotropy variance as spread
  ➢ Area roi allows Oval, Polygon, Freehand roi's
  ➢ Show grid to mark sub-roi's (useful when using Freehand roi)

Orientation Line plugin requires an image dataset in one of the following formats:

  ➢ a single dataset stack with 3 images (Anisotropy, Orientation, Average)
  ➢ a single dataset stack with 2 images (Anisotropy, Orientation)
  ➢ two datasets with single channel Anisotropy image(s) and single channel Orientation image(s)
  ➢ a dataset with only Orientation image(s)

In the cases where Anisotropy or Average image is not available their values default to max. When using other software than OpenPolScope for computing Anisotropy and Orientation image please refer to the scaling convention used below.

Orientation Image scaling convention:
8-bit: 0 - 179; 179 pixel value corresponds to 179°
12-bit: 0 - 1790; 1790 pixel value corresponds to 179° (1790/10)
14-bit: 0-7160; 7160 pixel value corresponds to 179° (7160/40)
16-bit: 0 - 17900; 17900 pixel value corresponds to 179° (17900/100)

Anisotropy Image scaling convention:
8-bit: 0 - 254; 255 reserved for values above ceiling value
12-bit: 0 - 4094; 4095 reserved for values above ceiling value
14-bit: 0-16382; 16383 reserved for values above ceiling value
16-bit: 0 - 65534; 65535 reserved for values above ceiling value

Usage

The controls to enable the Orientation Lines as well its options (Specs) are placed conveniently on the dataset window itself shown below in the red rectangle.
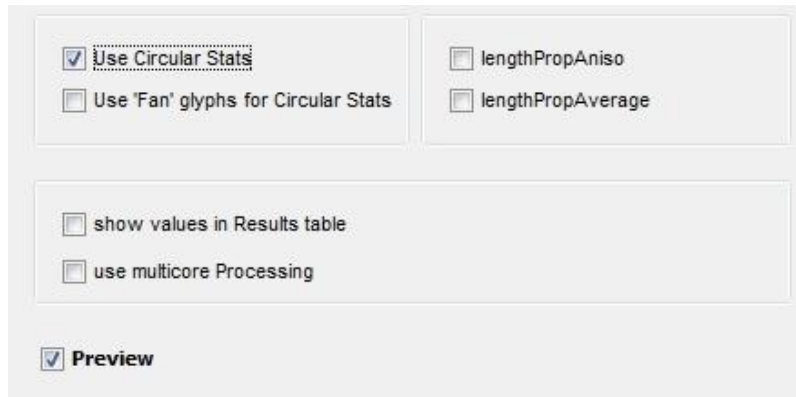

When Orientation Lines is run as an ImageJ plugin on an open image the Specifications dialog is presented from where the lines can be applied on the data.
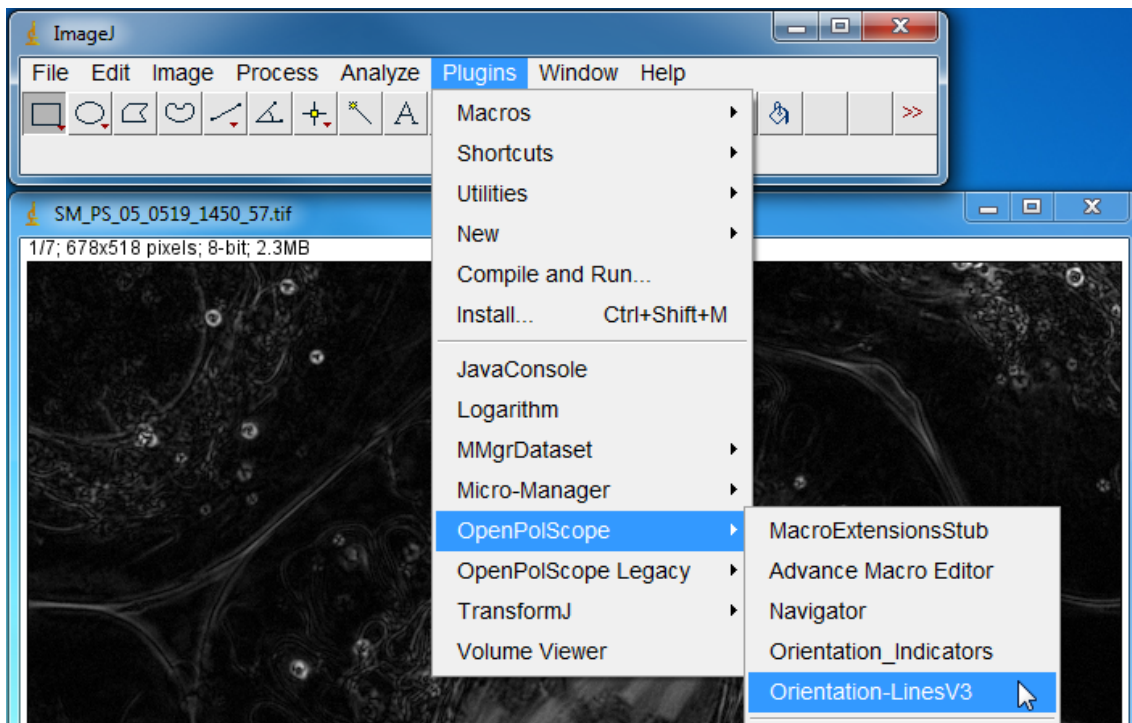
Select a roi selection (area selection - rectangle, oval, polygon or freehand) or dont to use the full image. Orientation Lines controls are located conveniently on the dataset as Checkbox to enable/disable or via 'Specs' to display or change Specifications (Draw Orientation Lines dialog).
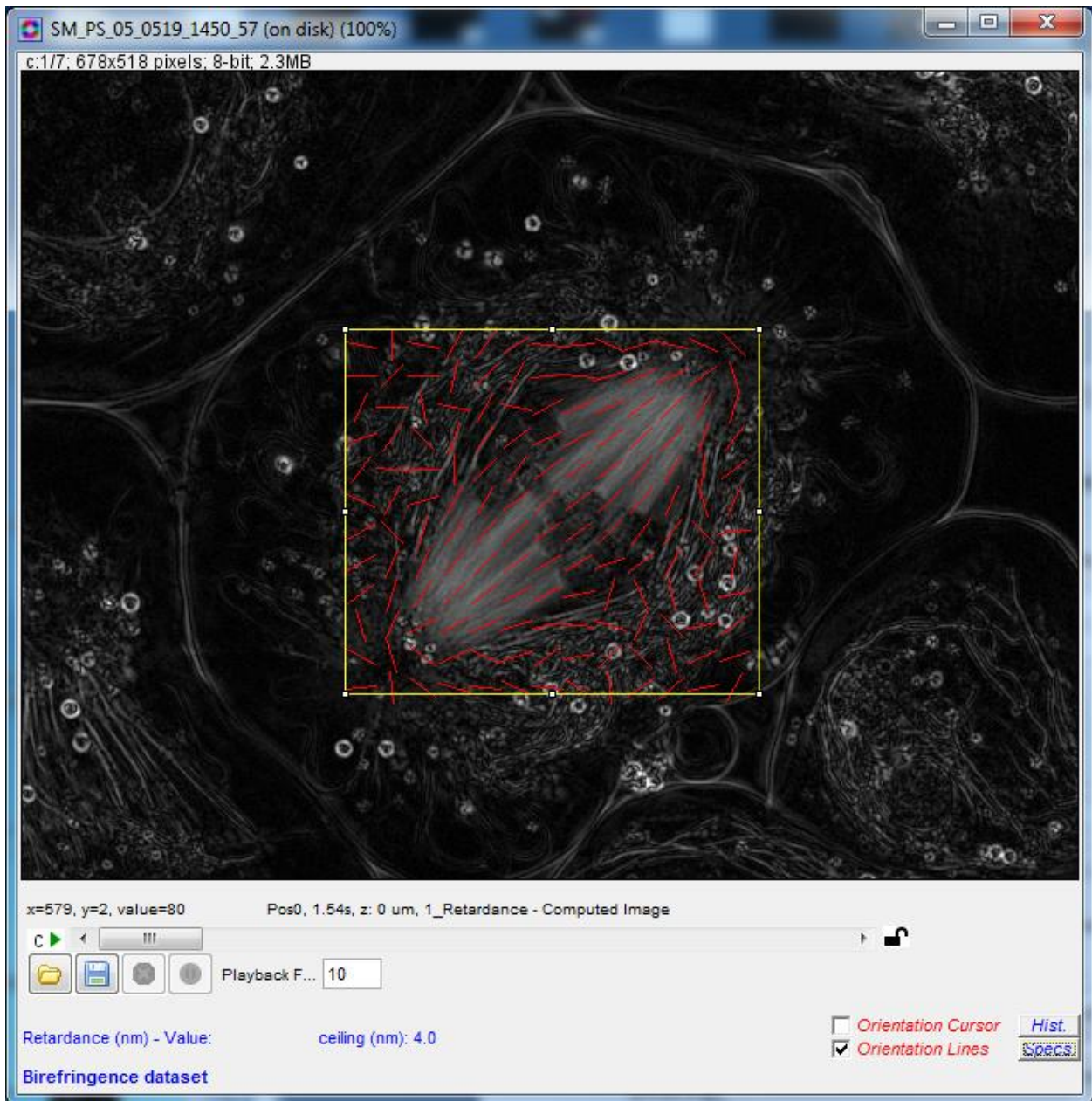
Controls available for Orientation Lines

Use Circular Stats: Enable to use or disable to use central pixel value of sub-roi

Fan glyphs: allows visualization of anisotropy with variance (fan spread)

lengthPropAniso: Length of line or glyph should be propotional to anisotropy

lengthPropAniso: Length of line or glyph should be propotional to Average

If both lengthPropAniso & lengthPropAniso are selected the Length is proportional to their product

Show values in Results table: Export values in terms of angle and anisotropy values computed from pixel values.

Use multicore Processing: uses 4 CPU cores simultaneously for processing by splitting the image into 4 equal quadrants.

Preview: applies the selected current options without closing the dialog and also redraws lines/glyphs automatically when changes are made in the dialog

General
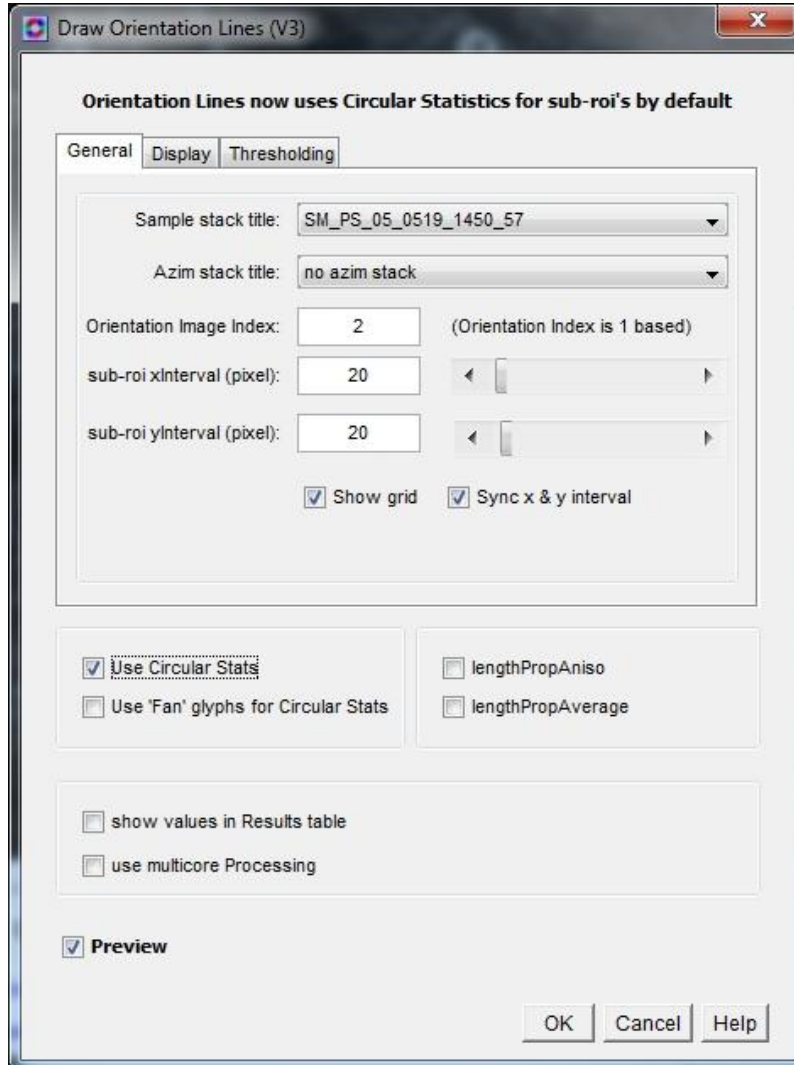
Sample stack will be the name of the current dataset.

Azim stack is a selection available if the Orientation image is in a different dataset.

Orientation image Index is the location of the Orientation image in the current stack. In Pol-Acquisition this is set as 2 however, when multi-modal data is acquired will shift accordingly and the new Index needs to be entered.

sub-roi intervals are the grids used for calculating over a sub-region within the Roi selection or the complete image.

Show grid displays the sub-roi grids

Sync x & y interval is an option to keep sub-roi's equal and use squares instead of rectangles.

Display
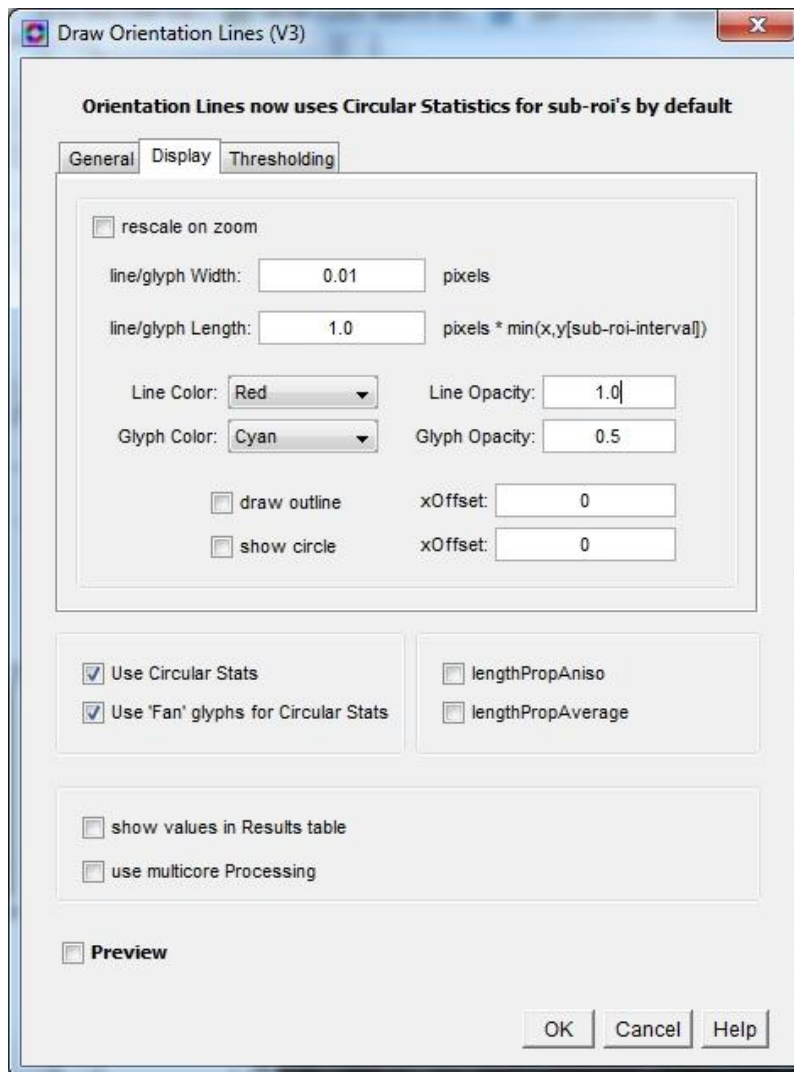
rescale on zoom: dynamically re-scale the sub-roi to provide optimum visuals based on image magnification.

line/glyph Width: width of the display line (allows sub-pixel resolution) - default is 0.01

line/glyph Length: length of the display line (the final value is the product of the minimum side of the bounding sub-roi box) - default is 1

Color: color of the display line or glyph

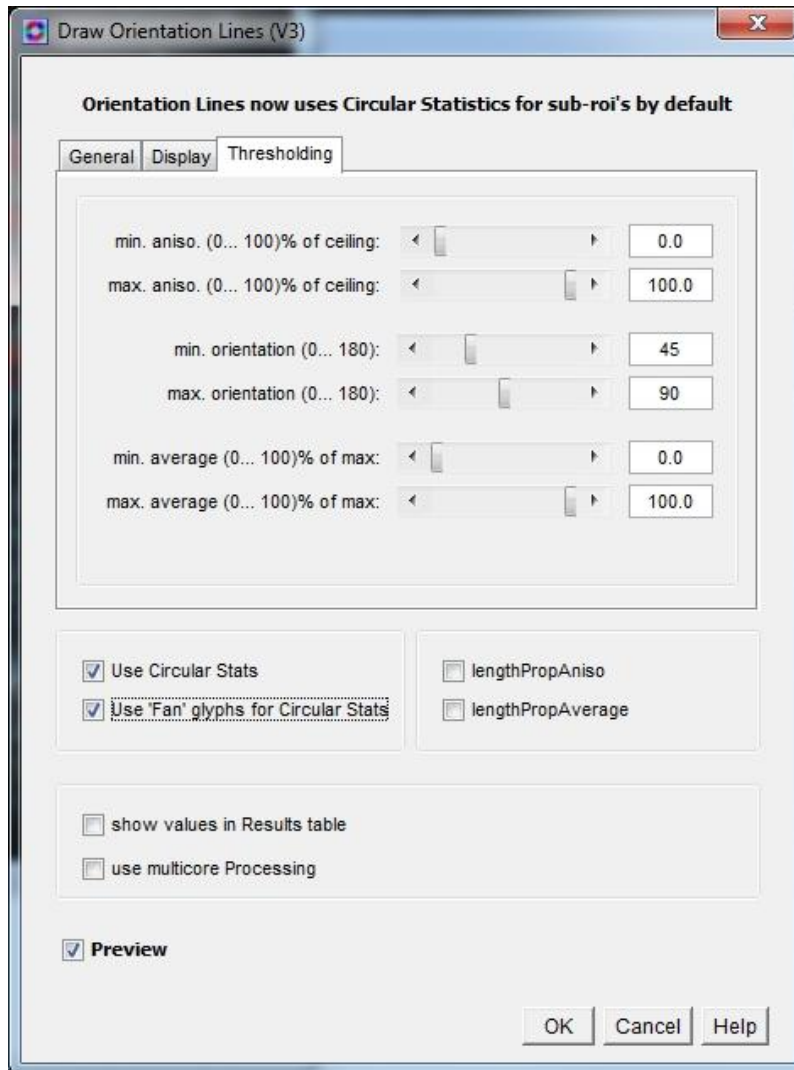Opacity: alpha value to deter-mine transparency of the line or glyph

draw outline:

show circle:

xOffset:

yOffset:

Thresholding

This sections allows thresholding of Anisotropy, Orientation and Average (where applicable) for display of Orientation Lines or Glyphs.

Anisotropy: Thresholding is based on percentage value. This provides consistency between 8,12,14,16 bit datasets.
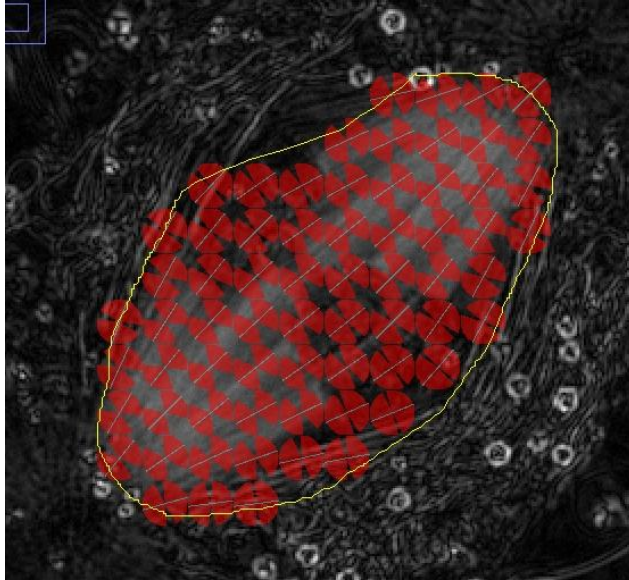
Orientation: Thresholding is based on 0-180 degree value. This provides consistency between 8,12,14,16 bit datasets.

Average: Thresholding is based on percentage value. This provides consistency between 8,12,14,16 bit datasets.
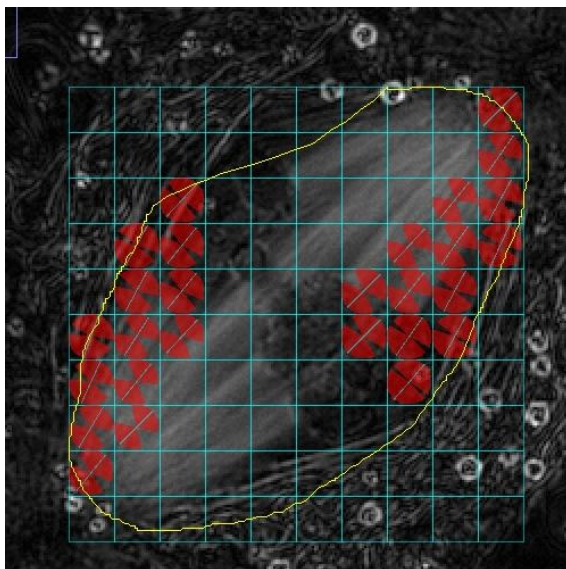
Visualization examples

Freehand selection with Circular Stats. and Fan glyphs:

Note: The area used for calculating the line or glyph when using Freehand or other type of Roi is the bounding box. The bounding box can be visualized using the 'Show grid' option - as shown below.

Freehand selection with Fan glyphs using show grid and angle threshold from 45-90 deg.:

Freehand selection with Circular Stats. Lines with show grid:
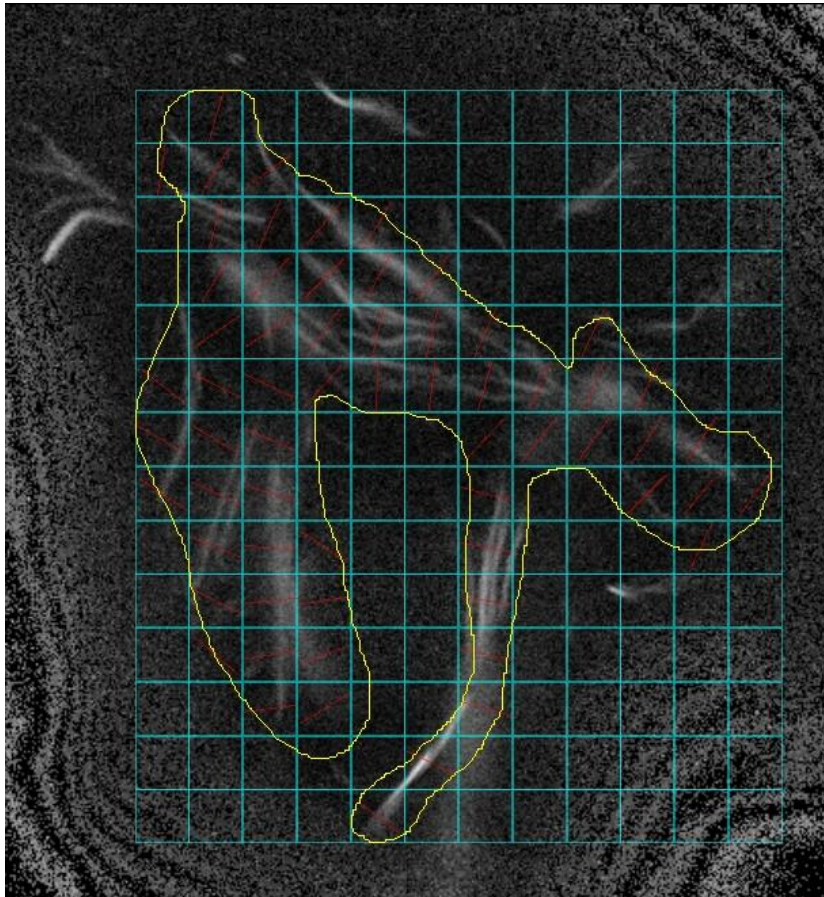
Roi assist macro:
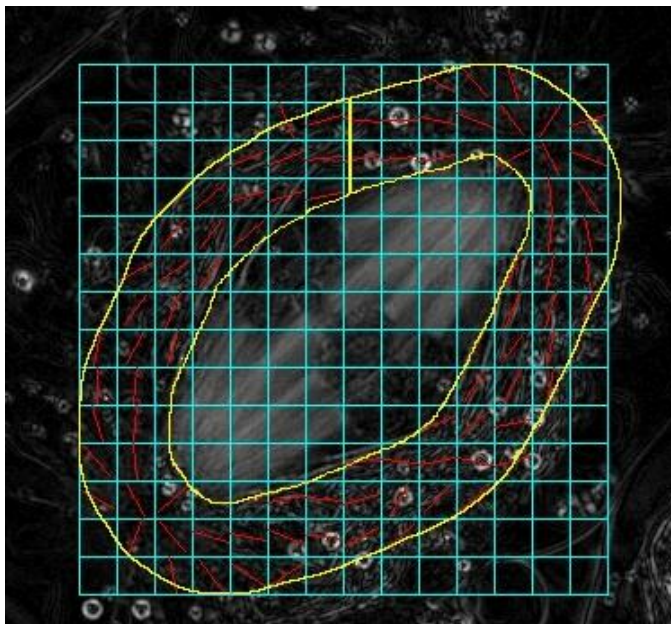
This macro creates a donut-band area selection from a regular area selection for plotting Orientation Lines.

It is useful where areas need to be omitted when plotting the lines and useful for boundary of objects, cell wall, etc.

Click Here to Download ImageJ macro

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