

Protocol for analyzing images with Open PolScope Pol-Analyzer (LC-MFM/Ludl)

1. Open micro-manager
2. Under ImageJ plugins → Open PolScope → Pol-Analyzer
3. **Under Data** → set data location path → designate user → designate a session → provide a description is needed
4. Check “overwrite original processed images” (**raw data will never be altered**).
5. Check “show overwrite confirmation message”
6. **Under Parameters** → open your sample of interest by clicking “open”.
7. Use “non background” for the background option unless a separate background image was taken.
8. All items under system should not be altered unless there was an issue with the system set up at the time of the acquisition. There are parameters set for the system upon image acquisition.
9. **Under fluorescence** → Anisotropy ceiling should be 1 pertains to $I_{\max} - I_{\min} / I_{\max} - I_{\min}$ → ratio ceiling should be 5 (pertains to I_{\max} / I_{\min}) → the difference ceiling is not relevant for our studies (pertains to $I_{\max} - I_{\min}$)
10. When using an LC the orientation reference is 0.0 (this is only a concern with a ludl wheel, in which the orientation reference will be calculated during GFP crystal calibration)
11. No of Raw images “5-frame”
12. Corrections
 - a. Select isotropic Region → in ImageJ draw a ROI in the cytosol of a cell in the field of view (this corrects for background bleaching between polarizer images)
 - i. In the drop down select “Yes compute new values”
 - ii. Check “Subtract Background fluorescence intensity”
 - iii. Check “Update Bleach Exponent”
 - iv. Check “Apply Instrument Correction”
 - v. Check “Update Instrument Correction”
 - vi. Once this is done click “Use Current ROI”, a BG should appear in the ROI
 - b. Select Specimen Region → Draw a ROI of the same size as the background ROI on the cytoskeletal structure in the same cell used for background in the field of interest (in this case a septin) (this corrects for bleaching within the structure of interest)
 - i. In the drop down select “Yes compute new values”
 - ii. Check “subtract specimen fluoresce intensity”
 - iii. Check “update bleach exponent”
 - iv. Click “Use Current ROI”, a SM should appear in the ROI
 - c. Only use “Apply Corrections in the Selected ROI only” if you only want to select for the ROI and now the whole image, this will rarely be used for Septin work.
 - d. Only use “Replace anisotropy and azimuth with averaged in the ROI” when you only want to assess ROI area. This will rarely, or never, pertain to us.
13. **Under Processing** → click fluorescence → if you want a colormap also click colormap → use exportview for display with azim lines on the image.
14. Options are only for convince and display processing

15. Logger is just for a log of the session
16. Once your image has been corrected you can click "Process"
17. Now in the drop downs under corrections select "Yes use the following values" until you move to a new set of samples and need to calculate new background and specimen bleaching.