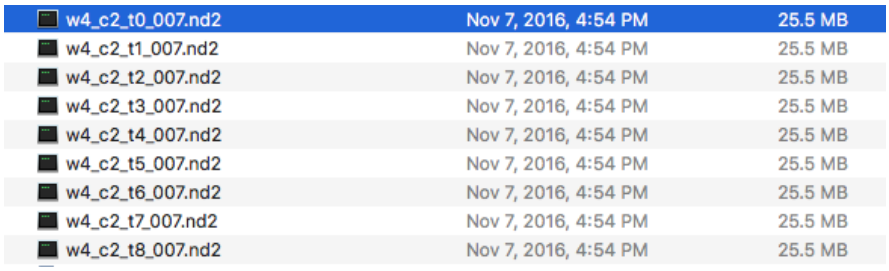
Polarized Order Detection Software

Tara Urner
Mattheyses Lab, University of Alabama at Birmingham
Last updated: 12/20/2017

The Polarized Order Detection Software package (PODS) was developed to measure pixel-by-pixel “Order Factor” in desmosomes [1].

Compatible input formats

1. **Nd2 files:** The program can process single-channel polarization .nd2 image stacks of four images (taken at 0°, 45°, 90°, 135° polarization angles in the x-y imaging plane). Larger image stacks are accepted, but only the first four images will be processed, and it is assumed that these images were taken at 45° intervals of rotation of polarization. The program can also accept two channel .nd2 images, currently only the first image in the sequence of the second channel is processed - it is saved as a .mat for later processing.
2. **Tiff files:** Tif files in the same arrangement as described above, excluding two color tifs, which cannot be processed at this time.
3. **Tiff and nd2 multiple files or timecourses:** Timecourses are placed in the corrected order based on string parsing of the identifiers “t0, t1, t2..” etc. Images without these identifiers or with non-sequential identifiers will not be processed correctly as timecourses. Multiple files that require the same flat field correction calibration can be processed at one time.



Input naming for timecourses

Optional Pre-processing

FindPBConstant.m can be used to find a photobleaching constant for the particular experimental setup and fluorescent protein of interest. The program can take single or multiple image stacks in either .tif or .nd2 format. To ensure that the constant represents the photodynamics of the fluorophore of interest, a masking routine is performed on the first image of the sequence. The same mask is applied to the last image and the constant is calculated with the following relationship [2]:

$$BleachExp = ln\left(\frac{< I_{first} >}{< I_{last} >}\right)/n_{exposures}$$

Images used to calculate the photobleaching exponent should have been acquired with the same exposure time as used experimentally and without any adjustments of beamline optics between frames. The polarization of the excitation light is not rotated while acquiring these images to simplify calculation of the photobleaching rate of the fluorophore.

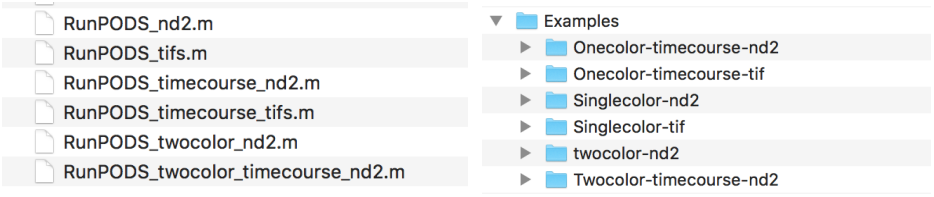
Image Analysis

PolarizedOrderDetection.m is the primary driver program for the analysis.

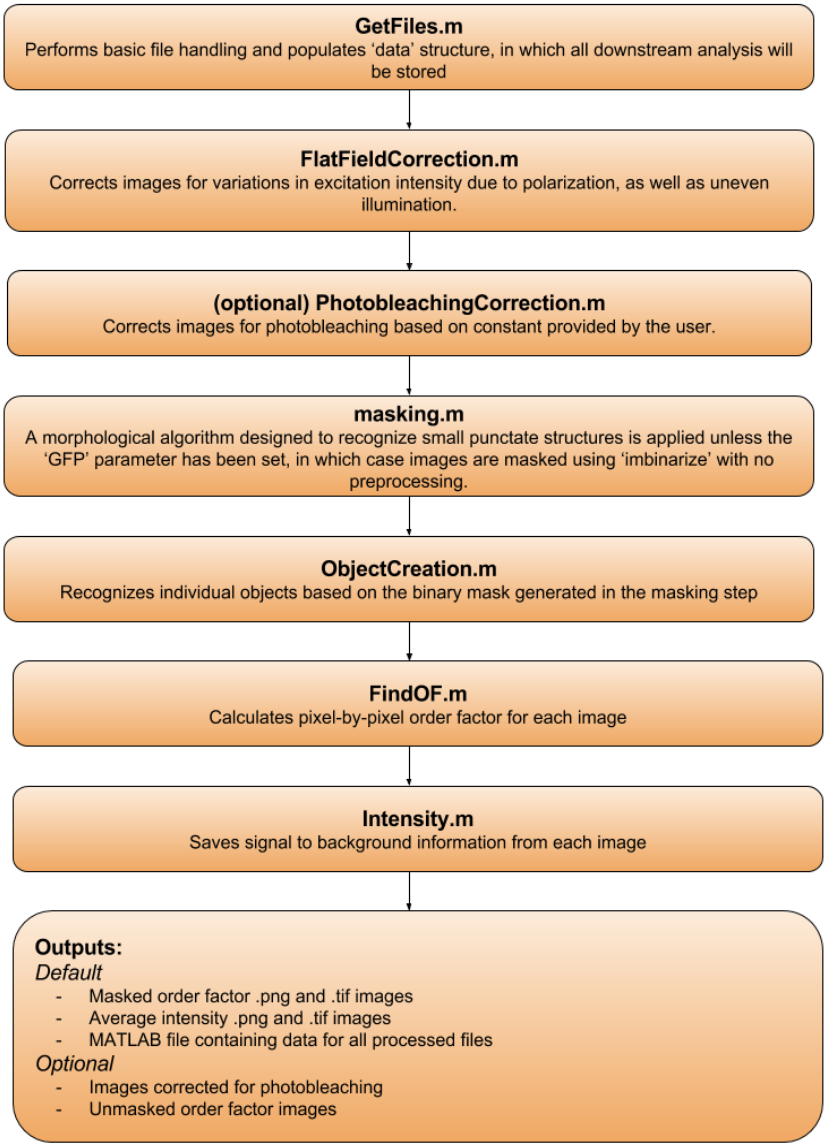
Input parameters:

Parameter name	**Type**	**Default**	**Description**
‘nd2’	logical	true	The default file type is a one channel nd2 polarization stack that is not part of a timecourse.
‘tifs’	logical	false	If the user wishes to process tifs, the ‘nd2’ parameter can be set to false, and the ‘tifs’ parameter can be set to true.
‘timecourse’	logical	false	Allows polarization stacks from sequential time points labeled by ‘t0, t1, t2,...’ to be processed, and (optionally) corrected for photobleaching, in order.
‘twocolor’	logical	false	For .nd2 files that have a single image in the second channel of the polarization stack. These images are extracted and written out as .mat files.
‘GFP’	logical	false	If true, this parameter changes the type of masking algorithm applied to images so that it correctly identifies an entire cell expressing a cytosolic fluorophore.
‘unmasked’	logical	false	Should be set to true if the user wishes to save order factor .png and .tif images without a mask.
‘pbcorrection’	logical	false	Optional photobleaching correction with known correction constant. If this parameter is set to true, the photobleaching constant provided by ‘pbconstant’ will be applied to correct each image.
‘pbconstant’	double	none	Correction constant for optional photobleaching correction.
‘debug’	logical	false	Optional parameter that writes function variables to the workspace for debugging purposes.

Several run files are provided for using different input formats with PODS, as well as example files for each format:



Analysis steps:



Adaptability

This program was designed to detect and analyze desmosomes. In the masking algorithm, desmosomes are recognized by their punctate nature and size at 60x magnification

(6.5 um^2 pixels; 108 nm/pixel). If the user wishes to adapt this program to measure the order factor of other structures the masking algorithm could be adjusted to pick out structures of interest. Alternatively, the 'unmasked' input parameter can be used to write out unmasked order factor images for additional downstream analysis.

Bibliography

[1] Bartle, Emily I, Tara M Urner, Siddharth S Raju, and Alexa L Mattheyses. 2017. "Desmoglein 3 Order and Dynamics in Desmosomes Determined by Fluorescence Polarization Microscopy." *Biophysical Journal* 113 (11): 2519–29. doi:10.1016/j.bpj.2017.09.028.

[2] DeMay, Bradley S., Naoki Noda, Amy S. Gladfelter, and Rudolf Oldenbourg. 2011. "Rapid and Quantitative Imaging of Excitation Polarized Fluorescence Reveals Ordered Septin Dynamics in Live Yeast." *Biophysical Journal* 101 (4): 985–94. doi:10.1016/j.bpj.2011.07.008.