BIOP PTS Instructions Manual

Installation & Protocol

T. Laroche O. Burri, A. Seitz

v1.1, June 2016

Summary

1. Installation
2. Interface
3. Settings
4. Performing PTS Analysis on a single image
5. Batch PTS Analysis
6. Summarizing Results
7. Extra notes

Installation

Prerequisites:

1. **ActionBar** plugin from Jérôme Mutterer
2. **BIOPLib Installer** and **BIOPLib.ijm**

Both of these can be installed via Fiji's "Update Sites":

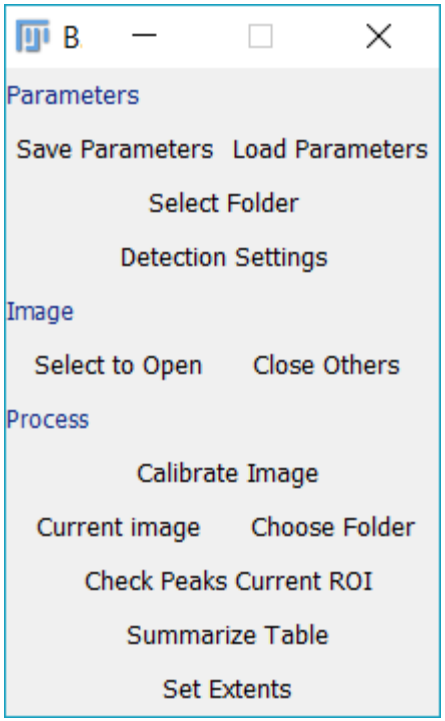
1. From Fiji, go to *Help > Update...*
2. Click "*Manage Update Sites*"
3. Check both '**IBMP-CNRS**' and '**PTBIOP**' update sites
4. Restart Fiji.

Installation:

1. Get the latest **BIOP_PTS.jar** at https://github.com/lacan/ijm-pts
2. Copy it inside the '**BIOP**' folder in your '**Plugins**' folder
3. Restart Fiji

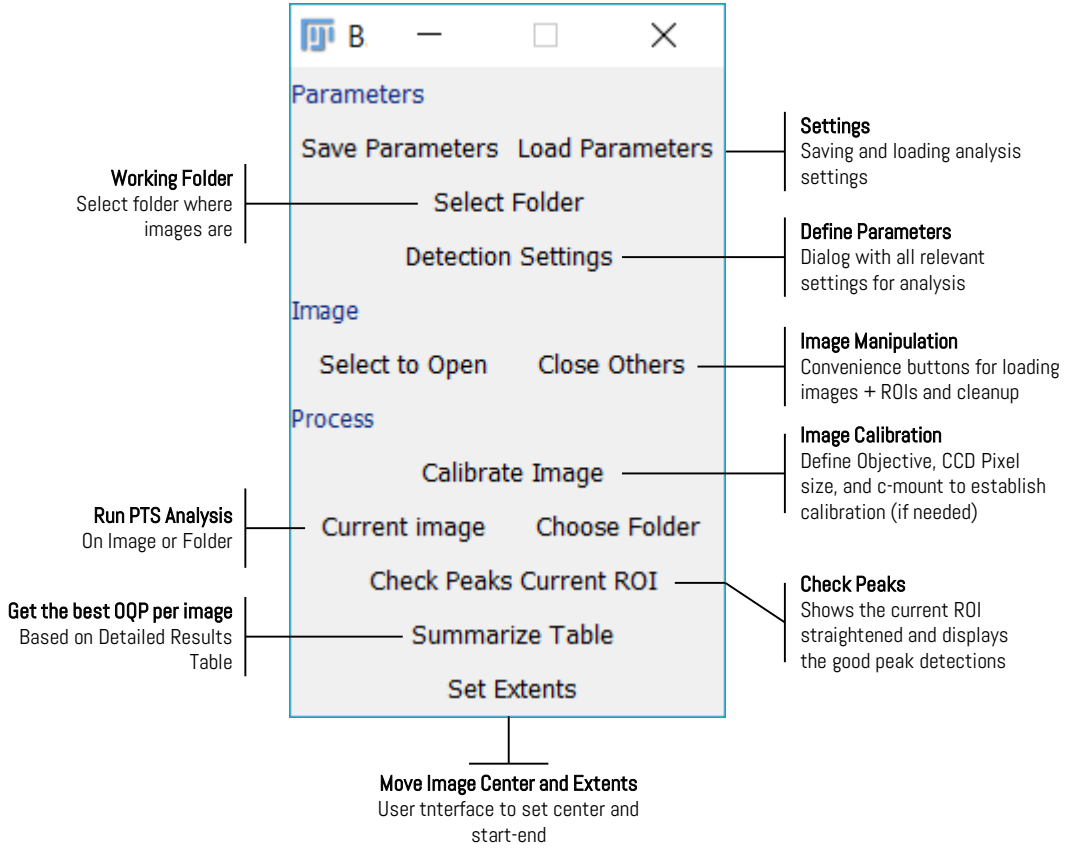
Testing:

Run the PTS by going to *Plugins > BIOP > BIOP PTS*



Interface

- The interface is provided as an ActionBar.



Settings

= Main settings =

Oversample Image	Rescales the image 2x in XY to allow for smoother detection by the peak detector
From Radius	Initial radius [in um] for the detection
Till Radius	Last radius [in um] for the detection
Increment	Step size [in um] between radii for analysis
Line Width	When the circle is straightened, interpolate the data around the circle by this much [pixels]
Distance Allowed Variation Percent:	If the calculated Peak-To-Peak distance lies within the theoretical Peak-To-Peak distance plus or minus this percentage, then it is considered as a valid distance

= Center Tuning =

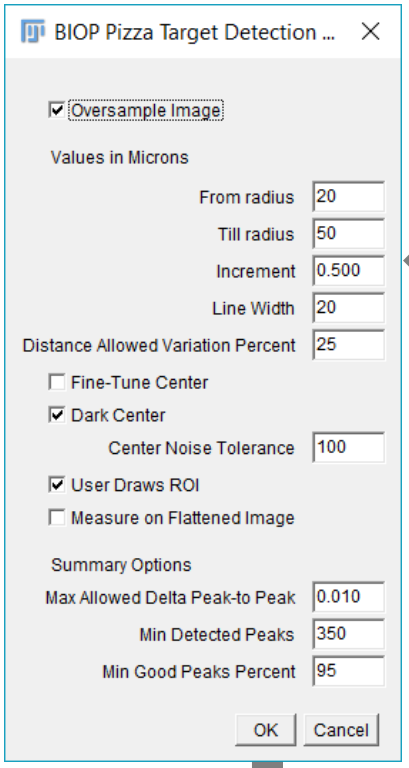
Fine-Tune Center	Attempt to correct for user-set center position by using "Find Maxima" with the parameters below
Dark Center	For V1 of the PTS, specifies whether the center point is dark on a light background.
Center Noise Tolerance	Noise tolerance for the "Find Maxima Plugin".
User Draws ROI	The user defined the center manually, otherwise it assumed the target is well centered.
Measure on Flattened Image	Performs pseudo-flatfield correction on the image before analysing

= Summary Options =

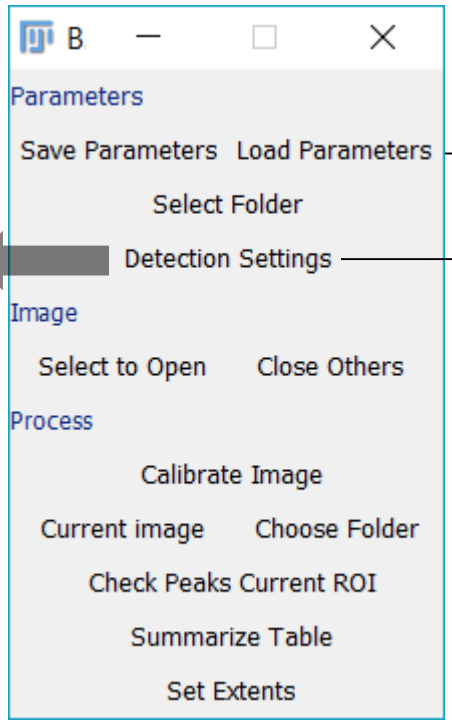
When all three criteria below are met, we consider that this is the OOP for the current image.

Min Detected Peaks	How many peaks should be detected as a minimum to consider we have found the maximum resolution.
Min Good Peaks Percent	How many among the peaks should have a distance that matches the theoretical distances
Max Allowed Delta Peak-to Peak	How much variance should we allow in the detected positive good distances

You can find a setting file with sensible defaults to get started with at **https://github.com/lacan/ijm-pts**

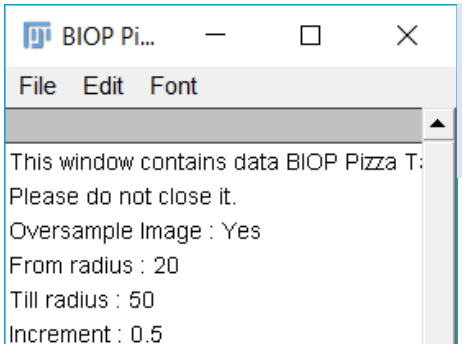


Clicking **'OK'** generates a text window that should be kept open



Settings
Saving and loading analysis settings

Define Parameters
Dialog with all relevant settings for analysis

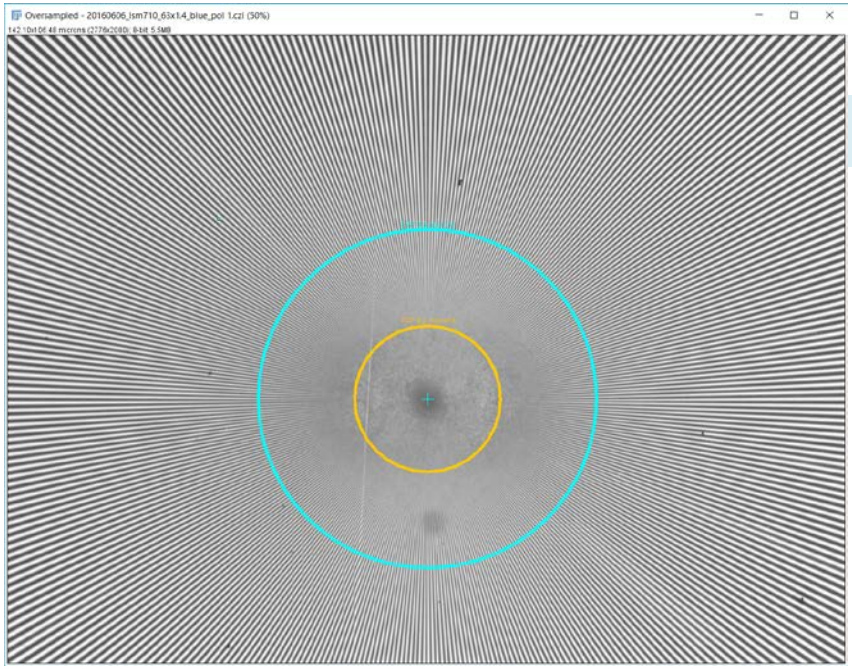


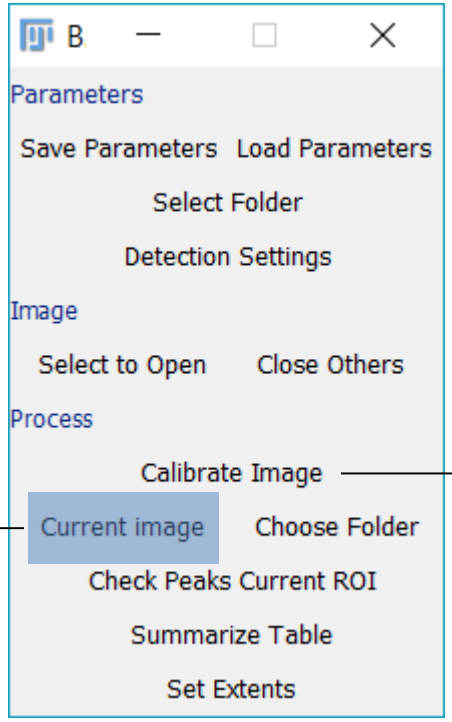
You can save these settings for use next time by clicking on "**Save Parameters**"

Single Image Workflow

After defining the PTS' settings and opening an image.

1. Ensure image calibration is correct
2. Click on "**Current Image**"
3. If "*User Draws ROI*" is set, you will be presented with an interface to define the center and extents.



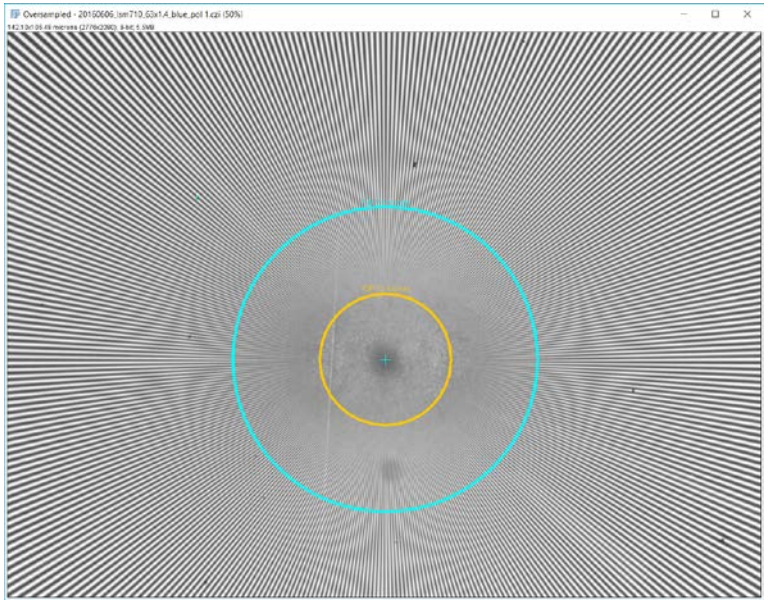


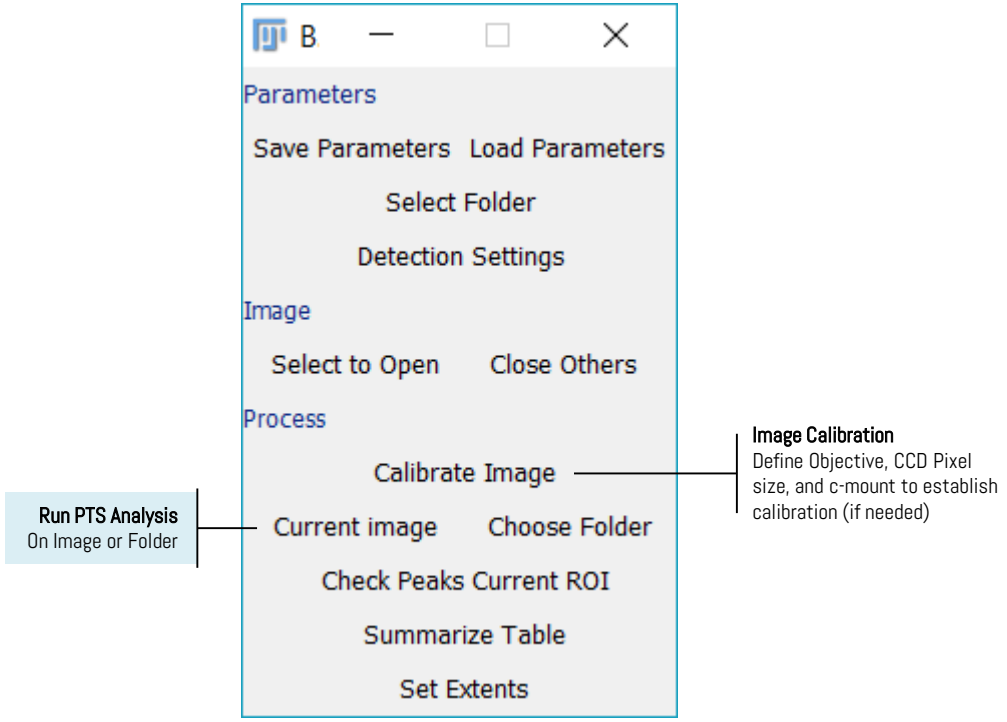
Run PTS Analysis
On Image or Folder

Image Calibration
Define Objective, CCD Pixel size, and c-mount to establish calibration (if needed)

Single Image Workflow – Manual Extents

1. Hover over the center cross with the mouse and wait until it changes color.
2. Left click and hold to drag the PTS center
3. Do the same for the two circles that represent from where till where the analysis should be performed





Once the extents are set, right-click to begin the analysis

Single Image Workflow – Results

A Result Table called "**Pattern Detector Detailed Results**" is created with all relevant data:

Label
Image name

Circle Radius
Radius of the circle where OQP was measured

Oversampled
Whether the image was oversampled

Expected Mean Peak-to-Peak Distance
Theoretical P2P distance, calculated as $R \sin\left(\frac{2\pi}{360}\right)$
With R: Circle Radius

Mean Peak-to-Peak Distance
Calculated P2P distance averaged from all "good" detections

StDev
Standard Deviation of all good P2P distances

Expected FWHM, FWHM
The full with at half maximum taken as 2/3 * P2P

Total Peaks
Total number of peaks detected by 1D Maxima finder

Good Distance %
The amount of peaks that had a P2P distance within bounds defined by "Variation Percent"

Pattern Detector Detailed Results

File Edit Font

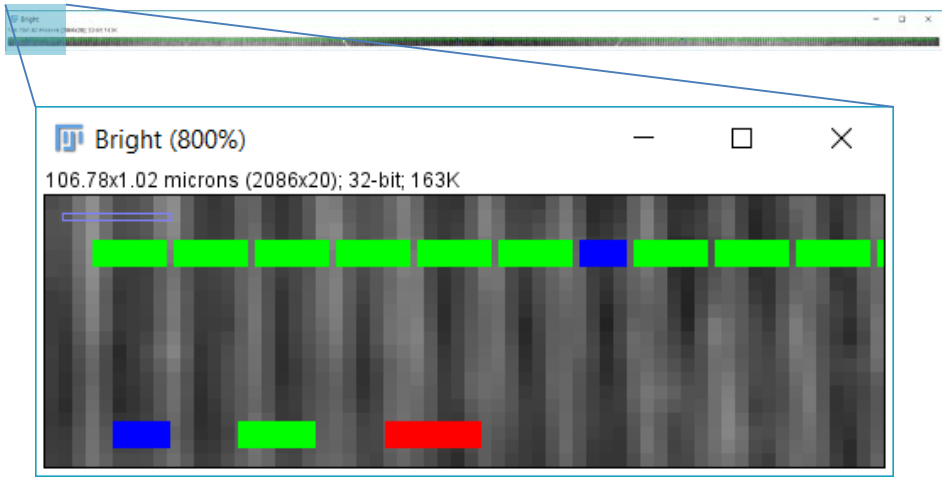
	Label	Circle Radius [microns]
1	20160606_lsm710_63x1.4_blue_pol 1.czi	12
2	20160606_lsm710_63x1.4_blue_pol 1.czi	12.500
3	20160606_lsm710_63x1.4_blue_pol 1.czi	13
4	20160606_lsm710_63x1.4_blue_pol 1.czi	13.500
5	20160606_lsm710_63x1.4_blue_pol 1.czi	14
6	20160606_lsm710_63x1.4_blue_pol 1.czi	14.500
7	20160606_lsm710_63x1.4_blue_pol 1.czi	15
8	20160606_lsm710_63x1.4_blue_pol 1.czi	15.500
9	20160606_lsm710_63x1.4_blue_pol 1.czi	16
10	20160606_lsm710_63x1.4_blue_pol 1.czi	16.500
11	20160606_lsm710_63x1.4_blue_pol 1.czi	17
12	20160606_lsm710_63x1.4_blue_pol 1.czi	17.500
13	20160606_lsm710_63x1.4_blue_pol 1.czi	18

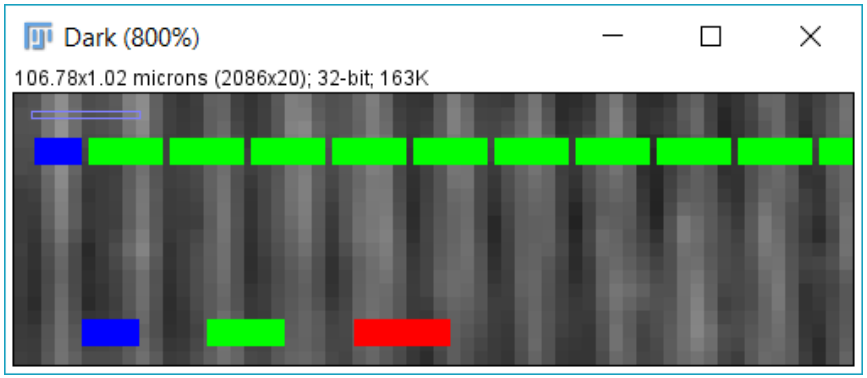
Max Consecutive Good Distances
How many consecutive good distances were detected

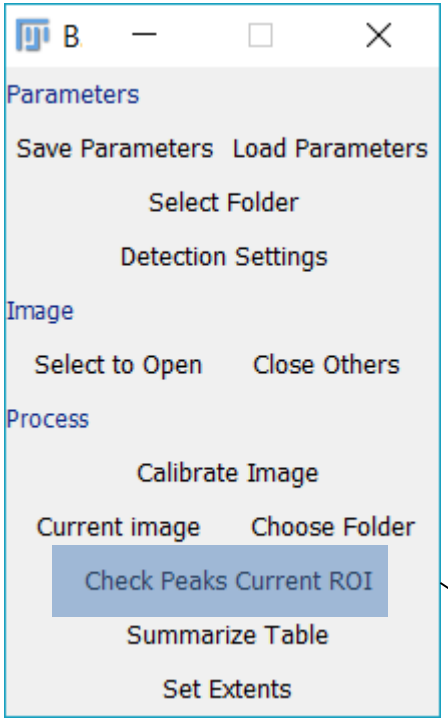
Michelson Contrast
Contrast metric defined as $\frac{I_{max} - I_{min}}{I_{max} + I_{min}}$
Where I_{max} and I_{min} are the averaged minimum and maximum intensities found by the 1D peak finder

Checking Analysis

After analysis, you can click on a circle in the ROI Manager and see how the analysis was performed on the bright and dark stripes







Check Peaks
Shows the current ROI straightened and displays the good peak detections

The color bars indicate what is considered as a "Good Peak to Peak Distance", as defined by the theoretical values plus or minus the "**Variation Percent**"

Anything shorter than blue is too short and longer than red is too long.

Batch Processing

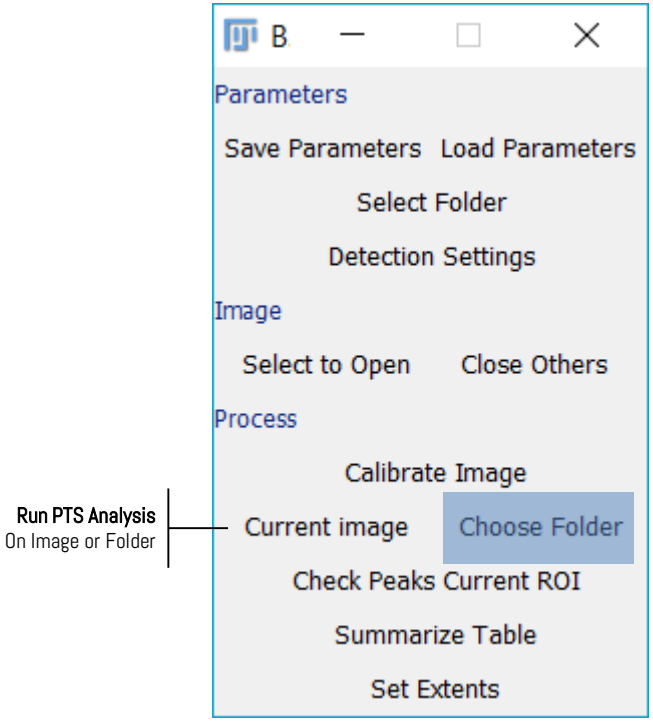
By clicking on "**Choose Folder**" you will be prompted for a folder.

After selection, each image will be opened and analysed in the same way as before.

Once an image is finished, it will be closed and the next one will open.

The results will all be appended to the table "**Pattern Detector Detailed Results**"

The ROI sets are saved for each image in a folder called "ROI Sets"

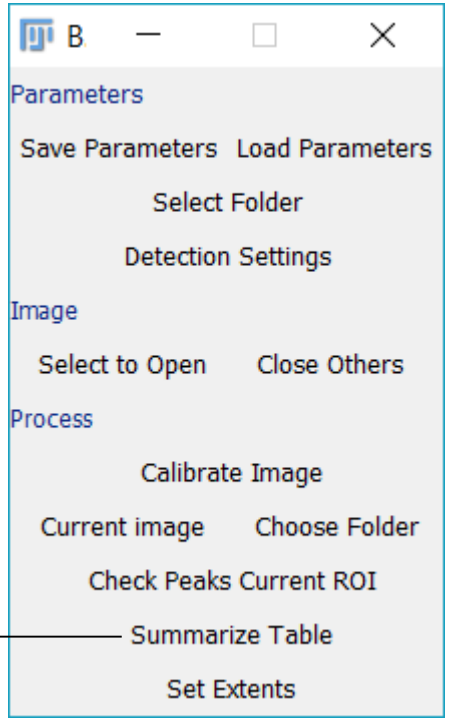


Summary Table

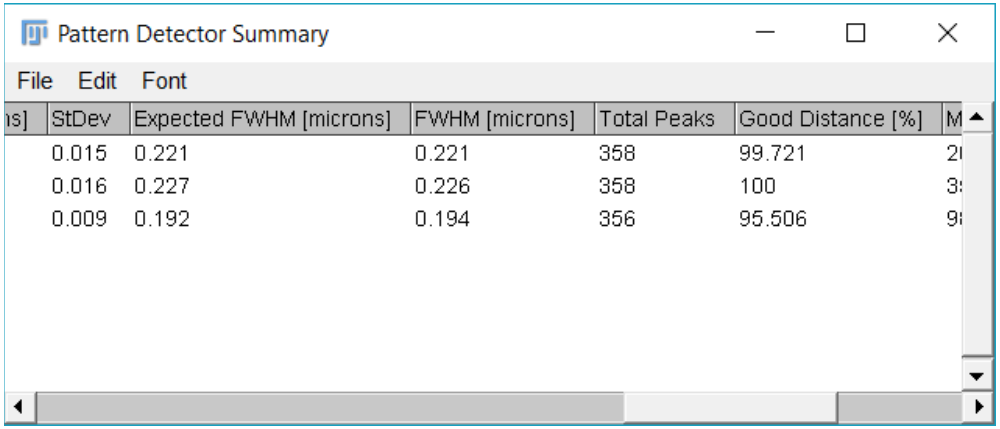
The current implementation displays all analyzed values. To find the one matching the OQP, one can use "**Summarize Table**"

A new table containing a single line per image is produced.

This is obtained by finding for each image, the first line in the Detailed results matching the "*Summary Options*" defined under "**Detection Settings**"



Get the best OQP per image
Based on Detailed Results Table



ıs]	StDev	Expected FWHM [microns]	FWHM [microns]	Total Peaks	Good Distance [%]	M
	0.015	0.221	0.221	358	99.721	2
	0.016	0.227	0.226	358	100	3
	0.009	0.192	0.194	356	95.506	9

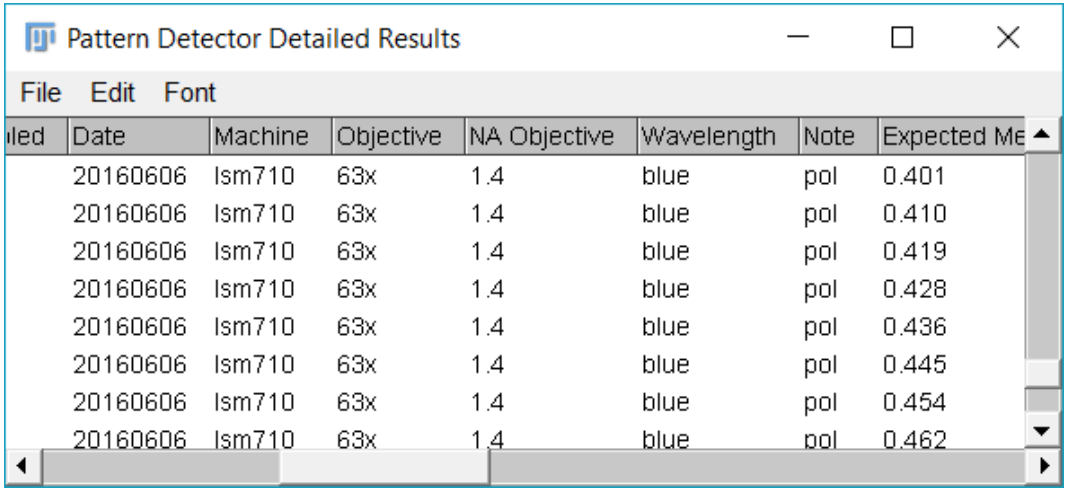
Note
To be able to summarize, the table called "**Pattern Detector Detailed Results**" must be open.

Extra Information From File Name

You can get extra information using proper filenaming convention

Example: 20150330_Zeiss-IN2_63x14_white_damage 1.lsm

Would export the following:
Date : 20150330
Machine : Zeiss-IN2
Objective : 63x
NA : 14
wavelength : white
Note : damage



ıled	Date	Machine	Objective	NA Objective	Wavelength	Note	Expected Me
	20160606	lsm710	63x	1.4	blue	pol	0.401
	20160606	lsm710	63x	1.4	blue	pol	0.410
	20160606	lsm710	63x	1.4	blue	pol	0.419
	20160606	lsm710	63x	1.4	blue	pol	0.428
	20160606	lsm710	63x	1.4	blue	pol	0.436
	20160606	lsm710	63x	1.4	blue	pol	0.445
	20160606	lsm710	63x	1.4	blue	pol	0.454
	20160606	lsm710	63x	1.4	blue	pol	0.462

These columns can help make sense of the data when compiling large amounts of PTS images