

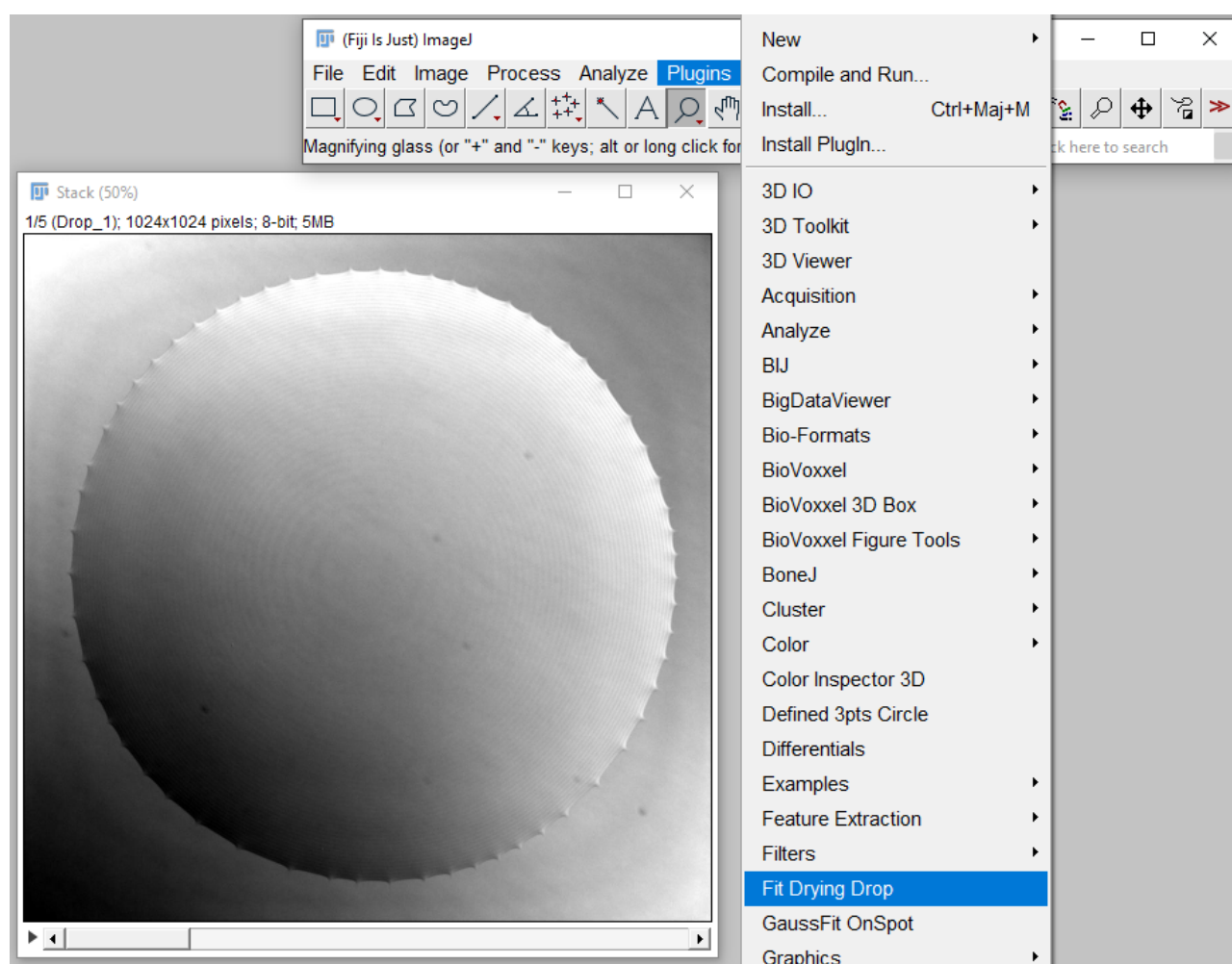
Tutorial: Fit Drying Drop plugin (v1.0)

Description: to propose a tool able to fit the perimeter of a drop (observed from above and whose meniscus is presumed very close to a circle). Some image pre-processings are used to enhance the raw image quality, and a set of outer and inner masks are applied in order to remove, as much as possible, false relevant points. **Results:** processed images stack, processed images, data tables.

Step 1: import the plugin in ImageJ/FIJI. Restart ImageJ/FIJI.

Step 2: build a stack from your images (in chronological order).

Step 3: select the Defined 3 pts Circle plugin.



Note: stack of images (here 5) of a drying 0.2 μl deionized water sessile drop acquired via a microscope (x10 objective, camera: 2048x2048 pixels, binning 2)

Step 4: fill in the fields with the appropriate information.

Fit Drying Drop: settings

----- Image Generality -----

Destination folder: D:\Folder\

Image(s) base name: FitDrop

Image format: tif

----- Image Time -----

Image time unit: ms

Duration of one frame: 80 in time unit

----- Image Scale -----

Image scale unit: µm

Reference length: 1024 in pixels

Reference real length: 1327 in scale unit

----- Outer mask Parameters -----

Outer mask offset value: 98.00 range: [0 - 100] %

Outer mask shrink value: 0.020 range:]0 - 1]

----- Inner mask Parameters -----

Inner mask offset value: 70.00 range: [0 - 100] %

Inner mask shrink value: 0.250 range:]0 - 1]

----- Image Background -----

☒ Image Background: white ?

----- Image Pre-processing(s) -----

☒ Subtract Background ☒ Anisotropic Diffusion

Rolling ball radius (subtract background): 50 in pixels: [10 - 200]

Smoothing (anisotropic diffusion): 1.000 std: [0.5 - 20]

OK Cancel

Image Background:

depending of the background, illumination of your images. In our case, despite the uneven illumination of the drop, we chose to check "True" (thus white background).

Image Pre-processing:

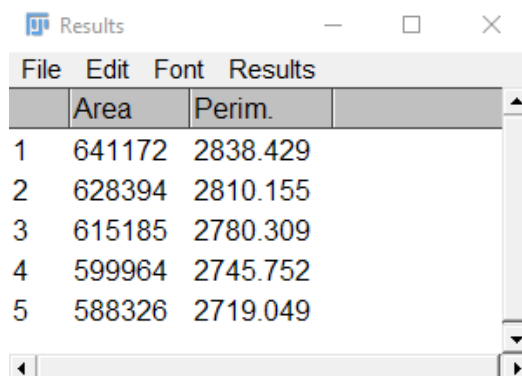
check if you want to apply on the stack a Subtract Background and/or an Anisotropic Diffusion.

Subtract Background is the image common processing tool available in the ImageJ's thumbnail Process.

Anisotropic Diffusion (5 iterations) is a 2D edge preserving de-noising filter¹. Plugin (v0.3) written by C. Rueden, J. Schindelin, M. Hiner and J-Y. Tinevez². In our plugin the user can set the strength of the smoothing parameter.

- 1 Tschumperle, D., & Deriche, R. (2005). Vector-valued image regularization with PDEs: a common framework for different applications. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 27(4), 506–517. doi:10.1109/tpami.2005.87
- 2 https://github.com/fiji/Anisotropic_Diffusion_2D

Results: two tables, a stack and N (the number of the images in the stack) images are generated.



File	Area	Perim.
1	641172	2838.429
2	628394	2810.155
3	615185	2780.309
4	599964	2745.752
5	588326	2719.049

"Results" contains the area (in pixel²) and the perimeter (in pixel) for each image of the stack.

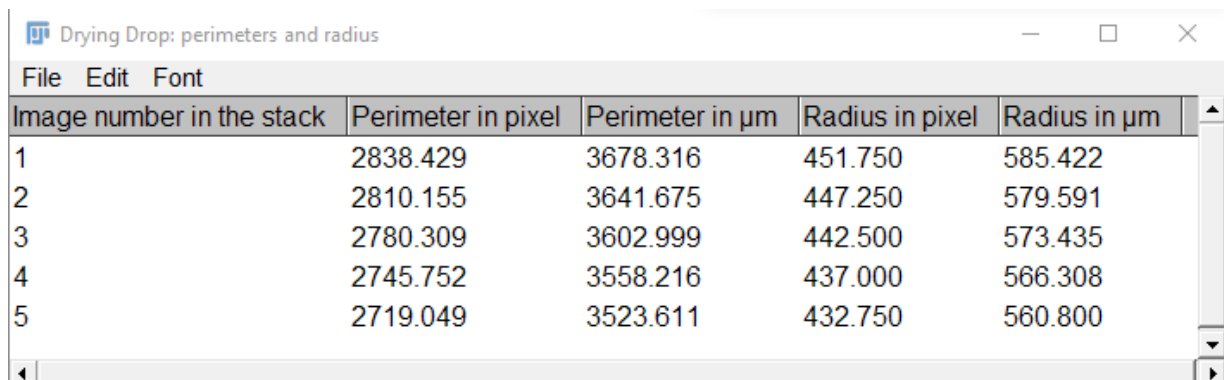
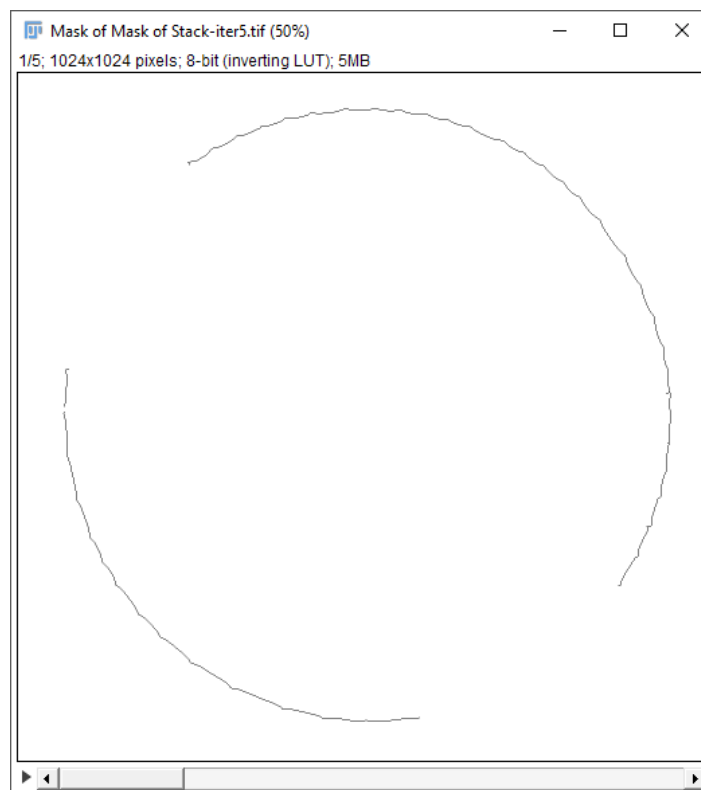


Image number in the stack	Perimeter in pixel	Perimeter in µm	Radius in pixel	Radius in µm
1	2838.429	3678.316	451.750	585.422
2	2810.155	3641.675	447.250	579.591
3	2780.309	3602.999	442.500	573.435
4	2745.752	3558.216	437.000	566.308
5	2719.049	3523.611	432.750	560.800

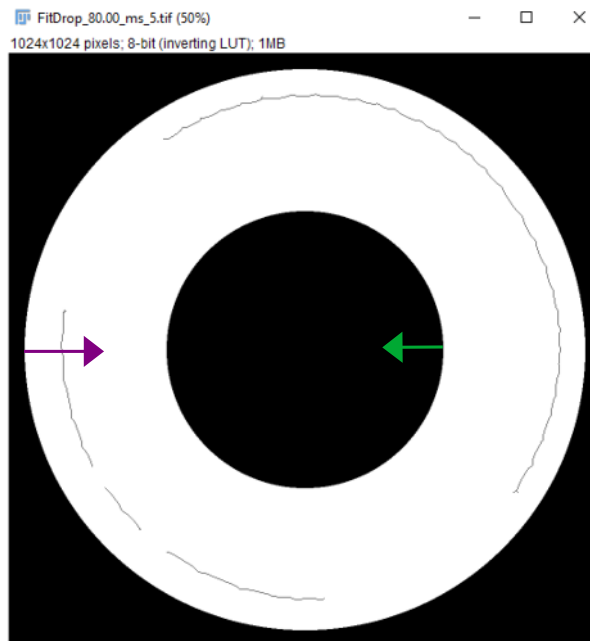
"Drying Drop" contains the perimeter and the radius of the fitted circle (both given in pixel and in the chosen unit scale) for each image of the stack.



As output, located in the Destination Folder, a new stack, containing the results of the processed input stack, is saved.



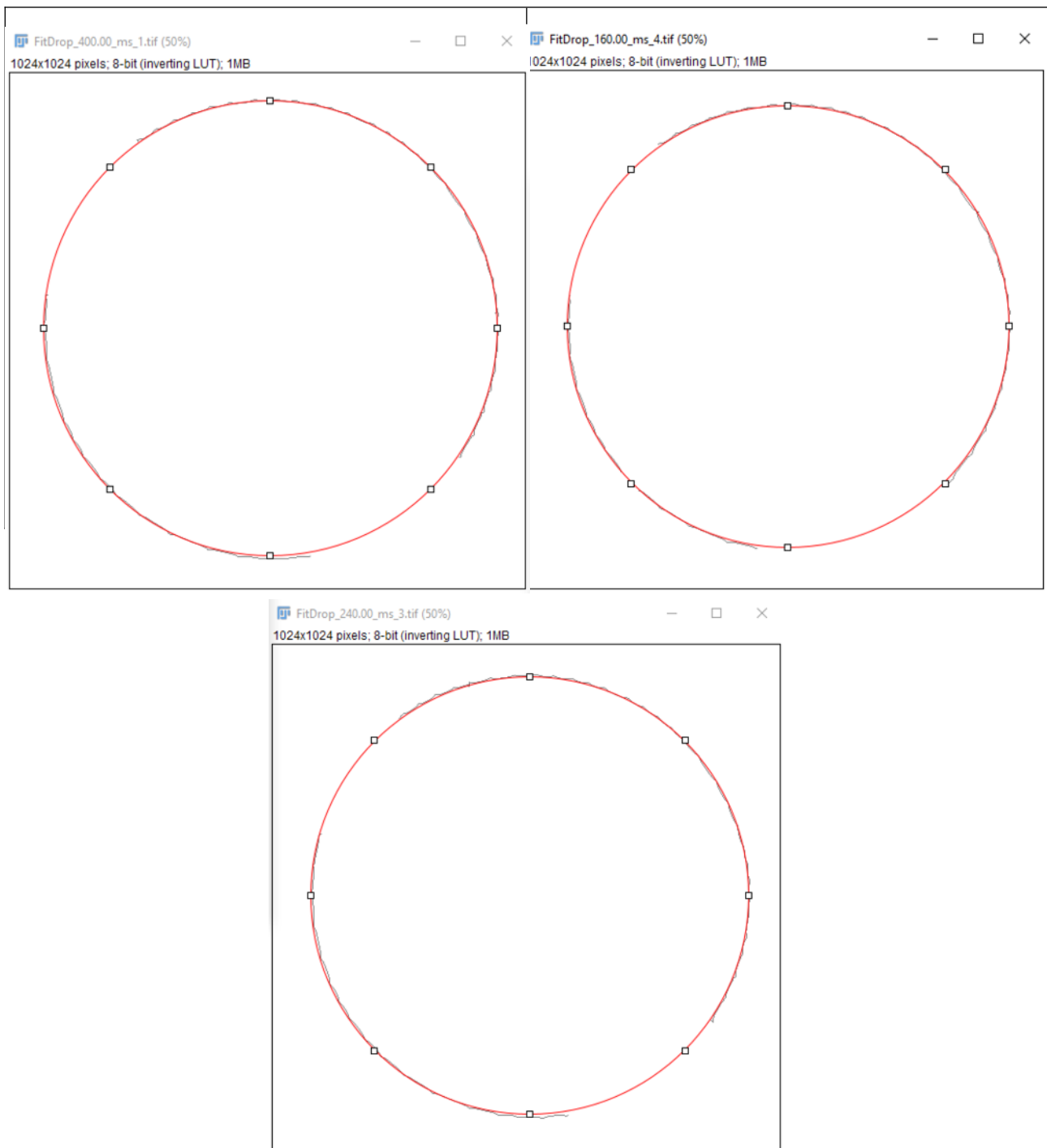
This image (n°1 in the stack) shows the outer and inner masks profiles applied on the first image of the stack. Accordingly to the Step 4, the outer mask offset is 98% (of the image's half width), the inner mask offset is 70%. **Remark:** the images have to be square.



This image (n°5 in the stack) shows the radius' evolution of the outer and the inner masks: from the first and the last image of the stack, the radius values change accordingly to the chosen shrink parameters (Step 4) and the total number of images in the stack. **Remark:** since the drop is supposed to shrink (evaporation) the radius of the drop should decrease...

Remarks: in these example, the masks are black (and not white as they should be) only to show their profiles: in the plugin the colour is set to remove as much as possible false relevant pixels. The higher the shrink parameter, the quicker the radius' shrinkage.

Additional outputs are provided in the Destination Folder: the images, from the processed stack, are individually saved. These images contain the fitted circle calculated from the meniscus detection.



Remark: the fitted circle appears in red on the images and is only shown if the image is saved and opened with ImageJ.

Plugin limitations: stack of 8 or 16 bits grey images; developed on ImageJ 1.54 K (may not work properly on earlier version).

If this plugin is used in your application and research, please reference it in your paper.