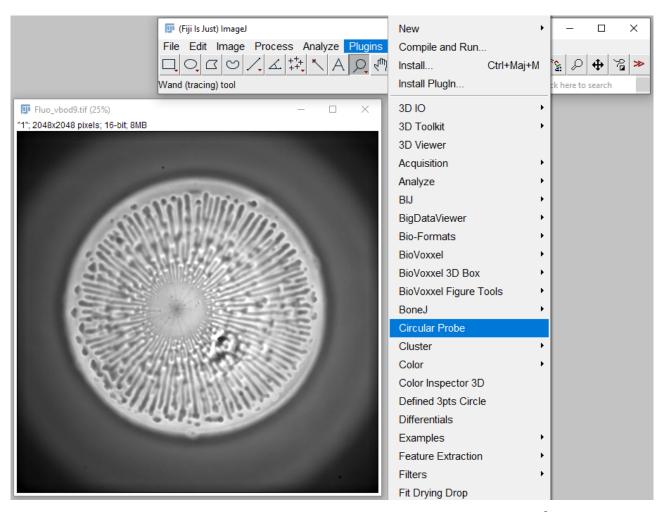
Tutorial: Circular Probe plugin (v1.0)

Description: to propose a tool able to probe, on an image, the grey values along the perimeter of a circle (previously drawn on the image by the user). As option, you can smooth the image and thus reduce its noise: image pre-processings can be selected and adjusted via parameters. <u>Results:</u> one data table and one graph.

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

Step 2: open the image and then select the Circular Probe plugin.



Note: image (fluorescence) of a dryed 0.5 μ l deionized water + Bodipy ([C] = 1.0x10⁻⁶) sessile drop acquired via a microscope (x10 objective, camera: 2048x2048 pixels)

Step 3: fill in the fields with the appropriate information, then click OK.

🛃 Circular Probe: settings		×			
Image Generalities					
Destination folder:	D:\Folder				
Image base name:	Fluo_vbod9				
Image Scale					
Image scale unit:	μm				
Reference length in pixel:	1998	in pixels			
Reference real length in unit:	13000	scale unit			
Results in pixels: yes ?					
▼ Anisotropic Diffusion + Median filters ▼ Gaussian blur + Enhance contrast					
Iterations (Anisotropic Diffusion):	5	nbr: [3 - 15]			
Smoothing (Anisotropic Diffusion):	1.000	std: [0.5 - 20]			
Sigma (Gaussian blur):	2.000	std: [0.5 - 20]			
		OK Cancel			

Results in pixels: check "yes" is equivalent to fill in 1.0 in both the Reference length and the Reference real length fields.

Anisotropic Diffusion: is a 2D edge preserving de-noising filter¹, plugin (v0.3) written by C. Rueden, J. Schindelin, M. Hiner and J-Y. Tinevez². The user can set: the number of the iterations; the strength of the smoothing parameter; the strength of the Gaussian blur.

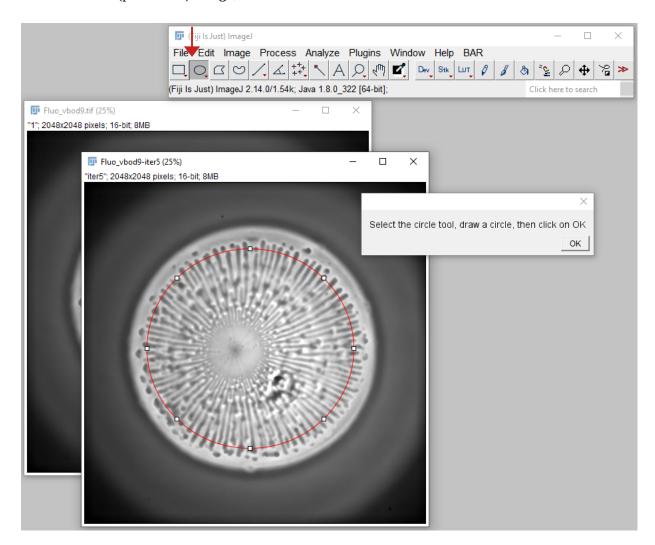
Median filter, Gaussian blur, Enhance contrast: are the image common processing tools proposed in the ImageJ's thumbnail Process.

Remark: the image processing generates a new image on which the probe circle must be drawn (see Step 4).

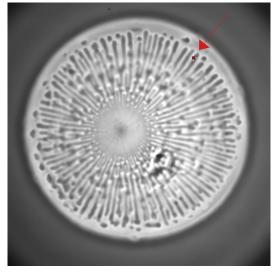
¹ Tschumperle, D., & Deriche, R. (2005). Vector-valued image regularization with PDEs: a common framework for different applications. IEEE Transactions on Pattern Analysis and Machine Intelligence, 27(4), 506–517. doi:10.1109/tpami.2005.87

² https://github.com/fiji/Anisotropic_Diffusion_2D

Step 4: select the "Oval, Elliptical or Brush selections" tool on the ImageJ banner, draw a circle by hand on the new (processed) image, then click OK.



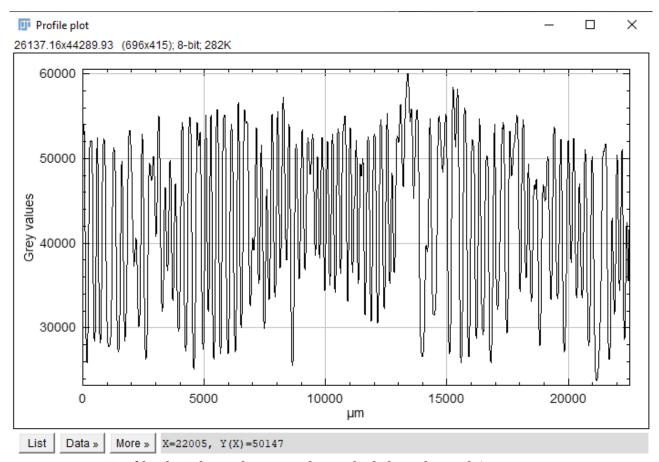
Remark: since the circle is drawn by hand, the minor and minor axes are slightly different \rightarrow the radius of the circle, used for the probe, is equal to the average between the minor and the major axes.



On the image, the probe is embodied by a red spot. This spot scans the image along the circle. **Results:** one table and one graph are generated.

🕎 Grey values Table						
File Edit Font						
Increments	Steps in µm	Abscissas in µm	Ordinates in µm	Grey values		
0	0.000	3676.176	3679.320	49322		
1	6.507	3682.683	3672.822	51140		
2	13.013	3689.189	3666.353	52484		
3	19.520	3695.696	3659.914	53338		
4	26.026	3702.202	3653.504	53803		
5	32.533	3708.709	3647.124	54007		
6	39.039	3715.215	3640.773	54029		
7	45.546	3721.722	3634.450	53883		
Q ↓	52 052	3728 228	3628 157	53527		
1						

"Grey values Table" contains the coordinates (in the scale unit) for each point, probed along the circle, and its grey level value.



"Profile plot" shows the grey value probed along the circle's perimeter.

Plugin limitations: 8 or 16 bits grey image; 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.