PombeProfiling Tools Documentation

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1. Introduction

The PombeProfiling tools are macros used to extract the fluorescence profile along the long axis of elongated cells such as *S. pombe* cells.These tools are used to make montages of cells or of patches.

2. Installation

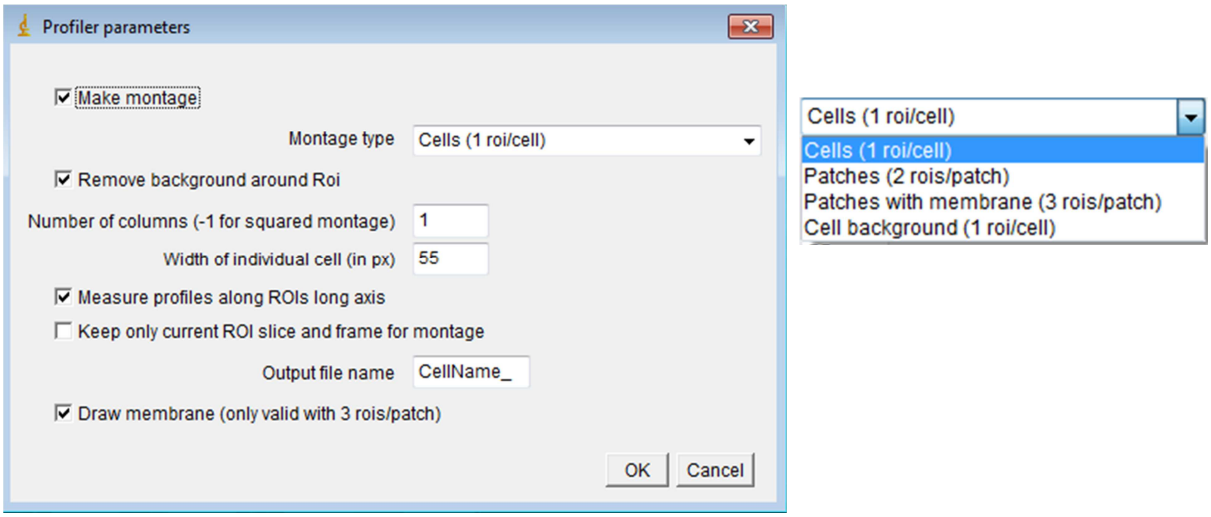
Copy the following files to an ImageJ macros subfolder

myProfilerToFile.ijm

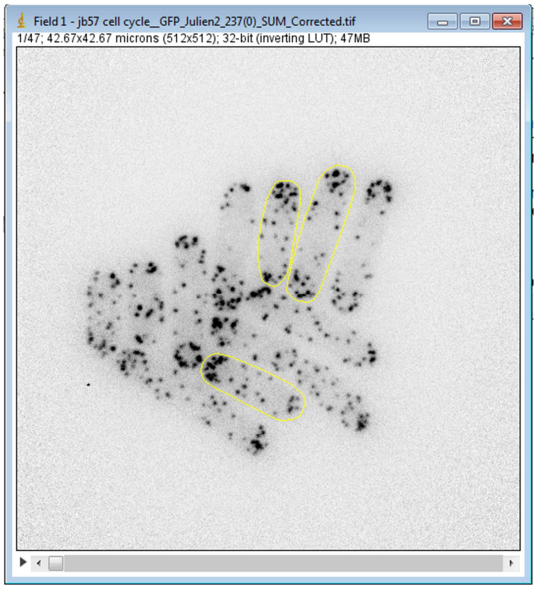
TransformPatchRoisetsForMontage.ijm

3. Cell profiles and montages

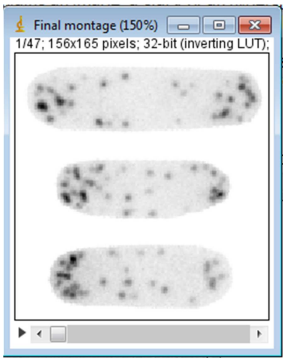
1. Open a z-projected file (or movie)
2. Outline cells (or open an roiset that contains the cell outlines)
3. Run the macro “myProfilerToFile.ijm”
4. The following dialog window appears:



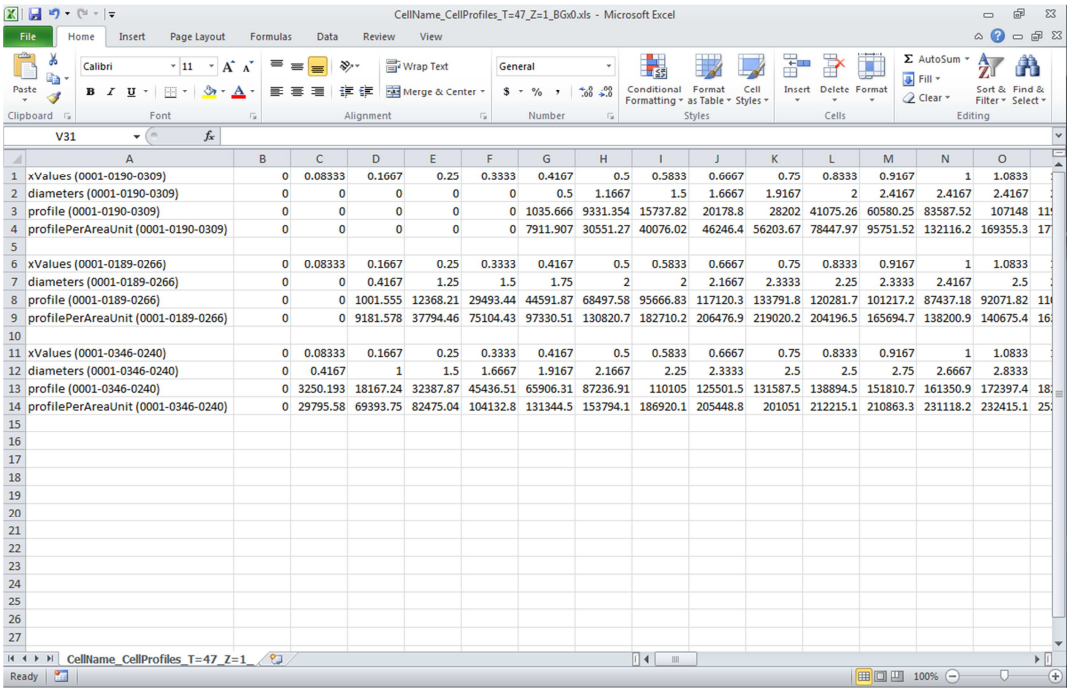
5. Check “Make montage” to also generate a montage of the cells
   Choose the first “Montage type“: “Cells (1 roi.cell)”
   For accurate cell profiles, check “Remove background around Roi”. The profiles will include only the fluorescence inside the cell. In the montage, the regions outside the cells is removed.
   Choose the “number of columns” for the montage, -1 for squared montages
   Choose the “width of individual cells” in pixel. The width is the size of the short axis of the cell.
   Check “Measure profiles along ROI long axis” to generate a file with the intensity profiles of the cells along their long axis
   Uncheck the box “Keep only current ROI slice and frame for montage” to generate a hyperstack montage; check the box to make a montage with the cells on the same frame and slice as their corresponding ROI (the montage will be an image).
   Choose the pattern for the “output file name”
6. The macro generates a montage of the cells and/or a data file containing the fluorescence profiles along the long axis of the cells.
   For the following movie, the macro generate the following montage and excel file for the 3 circled cells:



Montage:



Data file:



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	xValues (0001-0190-0309)	0	0.08333	0.1667	0.25	0.3333	0.4167	0.5	0.5833	0.6667	0.75	0.8333	0.9167	1	1.0833
2	diameters (0001-0190-0309)	0	0	0	0	0	0.5	1.1667	1.5	1.6667	1.9167	2	2.4167	2.4167	2.4167
3	profile (0001-0190-0309)	0	0	0	0	0	1035.666	9331.354	15737.82	20178.8	28202	41075.26	60580.25	83587.52	107148
4	profilePerAreaUnit (0001-0190-0309)	0	0	0	0	0	7911.907	30551.27	40076.02	46246.4	56203.67	78447.97	95751.52	132116.2	169355.3
5															
6	xValues (0001-0189-0266)	0	0.08333	0.1667	0.25	0.3333	0.4167	0.5	0.5833	0.6667	0.75	0.8333	0.9167	1	1.0833
7	diameters (0001-0189-0266)	0	0	0.4167	1.25	1.5	1.75	2	2	2.1667	2.3333	2.25	2.3333	2.4167	2.5
8	profile (0001-0189-0266)	0	0	1001.555	12368.21	29493.44	44591.87	68497.58	95666.83	117120.3	133791.8	120281.7	101217.2	87437.18	92071.82
9	profilePerAreaUnit (0001-0189-0266)	0	0	9181.578	37794.46	75104.43	97330.51	130820.7	182710.2	206476.9	219020.2	204196.5	165694.7	138200.9	140675.4
10															
11	xValues (0001-0346-0240)	0	0.08333	0.1667	0.25	0.3333	0.4167	0.5	0.5833	0.6667	0.75	0.8333	0.9167	1	1.0833
12	diameters (0001-0346-0240)	0	0.4167	1	1.5	1.6667	1.9167	2.1667	2.25	2.3333	2.5	2.5	2.75	2.6667	2.8333
13	profile (0001-0346-0240)	0	3250.193	18167.24	32387.87	45436.51	65906.31	87236.91	110105	125501.5	131587.5	138894.5	151810.7	161350.9	172397.4
14	profilePerAreaUnit (0001-0346-0240)	0	29795.58	69393.75	82475.04	104132.8	131344.5	153794.1	186920.1	205448.8	201051	212215.1	210863.3	231118.2	232415.1
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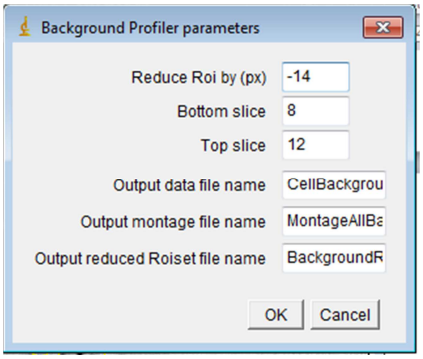
The data file contains 3 groups of data, one for each cell profile.
For each group,

the first row contains the positions x along the long axis,
the second row contains the cell diameter for each position x along the long axis
the third row contains the cumulative fluorescence at position x
the fourth row contains the fluorescence per area unit, ratio of the fluorescence at position x and the cell diameter at position x times x step

4. Profiles of background fluorescence

1 to 5. Same as previous section but pick “Cell background (1roi/cell)” as “Montage type”.
The background profiles can be estimated on projection images or on hyperstacks.

6. In the following dialog :



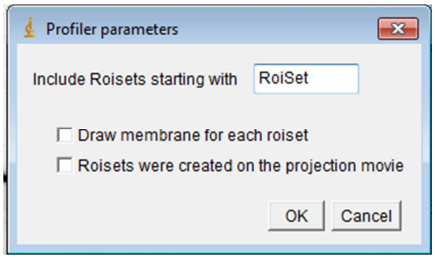
“Reduce Roi by (px)” sets the size the rois has to be shrunk to get the interior of the cell
The background fluorescence is estimated on the sum of the fluorescence between the “Bottom slice” and “Top slice”
The three bottom text boxes define the patterns for the names of the output files

7. The same kind of montages and data files as in previous section are generated. In addition the shrunk rois used for background estimation are saved.

5. Patch montages

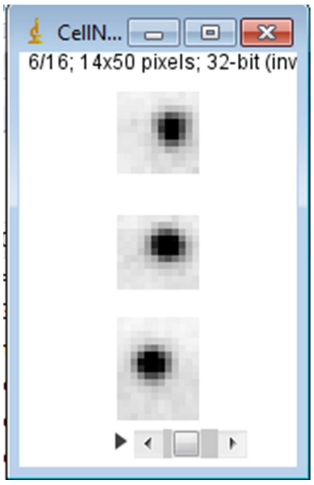
The same macro can be used to make montage of patches.
Each patch requires 2 or 3 rois. The first two rois are the first and last roi for the patch. The third one is optional and defines the position of the plasma membrane.
The macro “TransformPatchRoisetsForMontage.ijm” helps creating these rois from the roisets of patches used in the PatchTrackingTools.

When running the macro “TransformPatchRoisetsForMontage.ijm”, the following dialog box appears:



Roisets starting with the pattern in the box “Include Roisets starting with” will be transformed.
Check “Draw membrane for each roiset” to make montages where the patches are rotated such that the membrane is horizontal.
Check “Roisets were created on the projection movie” to remove the slice information from the rois.

To generate the montage, run the macro “myProfilerToFile.ijm” and choose for “Montage type” "Patches (2 rois/patch)" or "Patches with membrane (3 rois/patch)".



In the case of “Montage type” "Patches with membrane (3 rois/patch)", a file Angle.xls containing the orientation of the membrane for each patches.