Title : Automated version of FibrilTool ImageJ/Fiji macro [1,2]

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Brief description
In this automated version, user draw and save all ROI first and then let the macro compute automatically microtubule orientation on the set of ROI.

Requirements
.png 2D projection of one or several confocal stacks of fibrils (microtubules, actin…), and associated cell contours saved as a unique RoiSet.zip file on ImageJ/Fiji. Projection file and RoiSet.zip file must share the same name, for instance « 2015-08-20_LTi6B_A_T24h.png » and « 2015-08-20_LTi6B_A_T24h_RoiSet.zip » and be stored in the same working directory. See attached example of working directory (Working_dir.zip).
You can include in your directory .png images without any ROI (they will be skipped) and you can have single ROI (« 2015-08-20_LTi6B_A_T24h.roi »).
You can choose between .png, .tif and .tiff images. In Full_Log you now get the real name of the ROI instead of an incrementing number, after the name of the image.

Running the macro
1. Choose working directory (see Requirements).

2. Select Channels
As in manual version, select channels color of fibrils and channel color to draw mean orientation.

3. Macro is processing
Do not click on your screen, let the macro work. As there is not batch mode for the moment, clicking may interfere with the definition of the active window. This step is very slow, do not hesitate to run it overnight. If process abort for unknown reason, do not hesitate to run macro again (without modifying anything). This solve the problem in most of the cases.

During this step, macro takes each ROI of a given set one by one and computes fibril orientation. If there is several RoiSet files, macro loops over all given RoiSets.

4. Macro output
For each projection, the macro saves several files. A Log.txt file containing all informations on microtubule orientation (angle and quality) for ROI (e.g. each cell contour) of this projection (see [2] for details of Log.txt file content). Two .tif files on which are projected the orientation of microtubules over stack projection background or over black background. And two new RoiSet.zip files, containing ROI of orientation of fibrils (Mts stands for microtubules) with or without original ROI (e.g. cell contour).

References

1. Uyttewaal M, Burian A, Alim K, Landrein B, Borowska-Wykret D, Dedieu A, Peaucelle A, Ludynia M, Traas J, Boudaoud A, et al.: **Mechanical Stress Acts via Katanin to Amplify Differences in Growth Rate between Adjacent Cells in Arabidopsis**. *Cell* 2012, **149**:439–451.

2. Boudaoud A, Burian A, Borowska-Wykręt D, Uyttewaal M, Wrzalik R, Kwiatkowska D, Hamant O: **FibrilTool, an ImageJ plug-in to quantify fibrillar structures in raw microscopy**

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