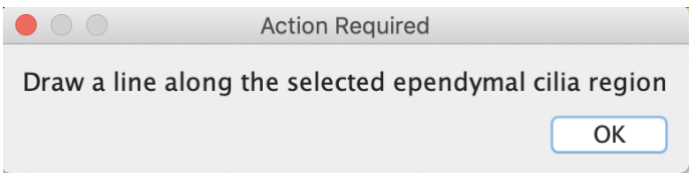
measureMultiCilia.ijm instructions.

"measureMultiCilia.ijm" is an interactive macro that helps measure the length and positioning of multicilia patches along neuroepithelial cell layers in immunostained brain tissue sections. The macro i) allows to select an epithelial region to be analyzed; ii) segments the patches (based on the fluorescence intensity signal of cilia markers); iii) represents the estimated lengths as lines along the multicilia apicobasal axes; iv) allows to fine-tune or delete the putative length lines; and v) delivers the results as an excel file containing the length of each patch as well as its distance respect to a reference origin, manually chosen by the user, e.g. the beginning of the ependymal cell layer.

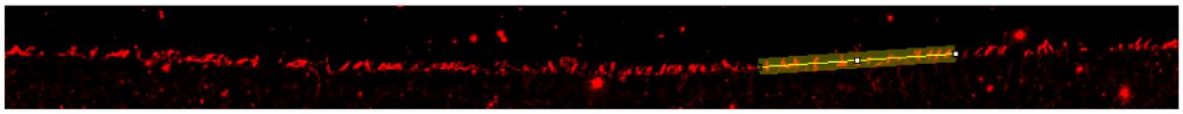
The macro can handle large images acquired as a mosaic that covers the whole neuroepithelial cell layer; it allows for selecting regions of interest (ROIs) containing multicilia patches and processing them in a sequential manner. A sample image is provided that has been acquired in a Leica Thunder Imager 3D Live Cell, equipped with a 100x (oil HCX PL-APO, NA 1.4-0.7) objective lens and simultaneously processed using the instant computational clearing (ICC) algorithm provided with the system. The original image has been acquired as a XYZ mosaic covering an ependymal layer extension of 2.5 mm and the maximum projection has been computed before using the macro. Due to excessive size, only a fragment of the image is provided for macro trial (Courtesy of Murielle Saade, IBMB). An arbitrary reference point should be used on this particular example for positioning measurements.

How to use

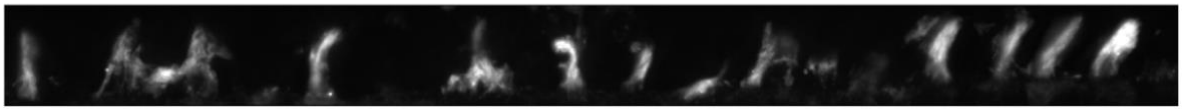
1. Open the file "measureMultiCilia.ijm" by drag and drop to the Fiji bar.
2. Open the image.
3. Annotate the x and y coordinates (in μm) that correspond to the reference origin of the neuroepithelial cell layer; then type them in the corresponding coordinates location at code lines 22 & 23.
4. Run the macro by hitting *run* at the script editor.
5. A dialog box will pop up asking to choose the results folder.
6. A message appears asking you to draw a line through several cilia patches (a). After hitting *OK*, the region will be duplicated in a new window (b).



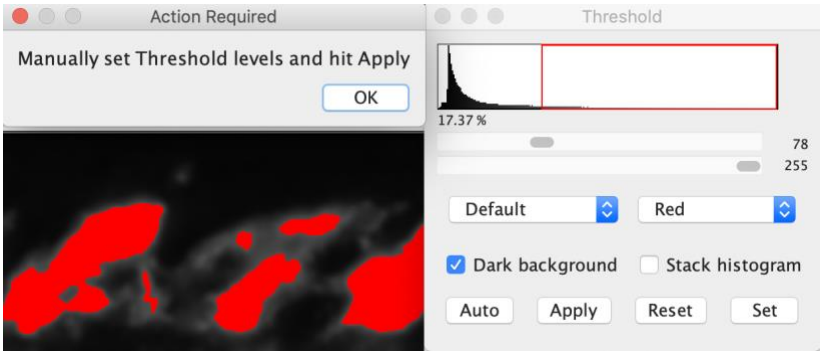
a



b



7. Adjust the threshold manually and click the *Apply* Button (c). A binary mask is obtained (d).

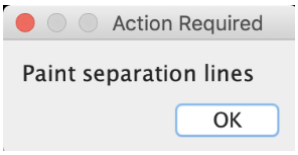


c

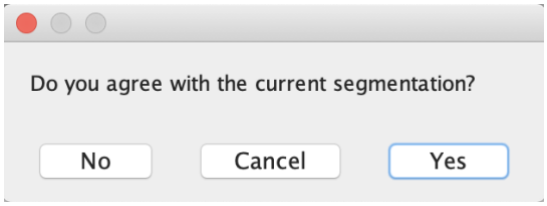
d

8. A new message “Paint separation lines” will ask you to draw lines between contiguous patches. This procedure can be repeated as many times as needed before hitting *OK* (e)

e

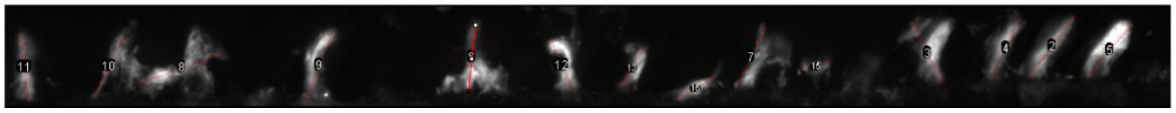


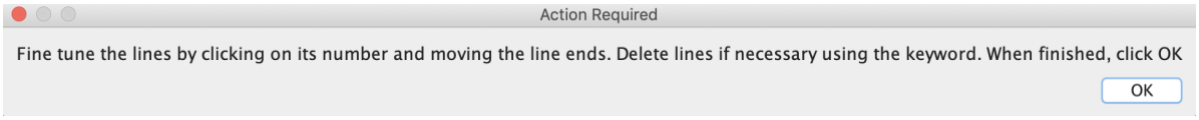
9. Next, the macro returns several objects onto the binary image together with the message “Do you agree with the current segmentation?” to either accept the segmentation or run some more splitting events.



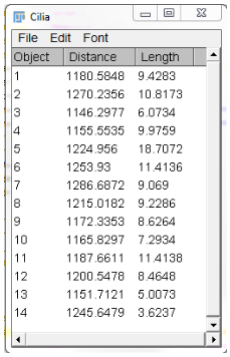
10. The Feret diameters of all segmented objects are measured and shown onto the duplicated fluorescence image (f). The lines can be fine-tuned manually to better adjust them to the shape of the multicilia patches, if required. To do so, click on the line number and modify the line end points. To delete a line, just select it and delete using the keyboard.

f





11. Finally, the length of the multicilia patches and their distance to the selected neuroepithelial layer's reference origin are shown in the results table and saved as an excel file.



Object	Distance	Length
1	1180.5848	9.4283
2	1270.2356	10.8173
3	1146.2977	6.0734
4	1155.5535	9.9759
5	1224.956	18.7072
6	1253.93	11.4136
7	1286.6872	9.069
8	1215.0182	9.2286
9	1172.3353	8.6264
10	1165.8297	7.2934
11	1187.6611	11.4138
12	1200.5478	8.4648
13	1151.7121	5.0073
14	1245.6479	3.6237