**FIND NETWORK EDGES WITH WATERSHED SEGMENTATION – 11/01/2021**

This Fiji macro was tested on HeLa and COS-7 cell cultures. It does not work for neurons.

**Input:** Thresholded fused images the DAPI channel and the phalloidin channel. Examples: B02\_th\_dapi.tif and B02\_th\_phalloidin.tif.

**Outputs:**

* cell\_measurements.csv: contains morphological measurements of the cells (area, circularity, longness).   
  Circularity = 4\*pi\*area/(perimenter^2). A circle has circularity 1.  
  Longness = longest axis of bounding box / shortest axis of bounding box.
* nuclei\_com.csv: contains the coordinates of the centres of mass (COM) of all nuclei.
* edges.csv: contains a list of which nodes are connected and which are not.
* <well>\_wts.tif: the segmented image, where each cell has a separate grey value.

**Code:** the Fiji macro 4\_findNetworkEdges.ijm inside the folder

CellContactNetwork > Fiji macros > HeLa processing.

**Backend dependencies:**

* Fiji plugin “Distance transform watershed” (embedded in MorpholibJ): <https://imagej.net/Distance_Transform_Watershed>
* Fiji plugin “marker-controlled watershed” (embedded in MorpholibJ):

<https://imagej.net/Marker-controlled_Watershed>

* Custom-built Fiji plugin “Network creator”. Find the .jar file in

CellContactNetwork > Java Plugins > Make markers

This plugin takes as input (1) the Dapi threshold and (2) a path to the output folder. It measures the nuclei COM, stores the measurements in nuclei\_com.csv and outputs an image with the same size as the fused image with white pixels on the coordinates of the nuclei COM.

* Custom-built Fiji plugin “Network creator”. Find the .jar file in

CellContactNetwork > Java Plugins > Network Creator

This plugin labels the connected components in a segmented image and outputs them in edges.csv.

**How to run it:**

Graphical user interface, text, application, email

Description automatically generated