Cell Segmentation from DIC Images - Tutorial

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Cell segmentation from DIC images relies upon the ImageJ Morphological Segmentation plugin, which is part of the MorphoLibJ[1] library.

Installation[2]:

1) Start FIJI updater by selecting *Help > Update…*

2) Click on *Manage update sites*[3] and activate the *IJPB-plugins*[4]

3) Close and apply changes

4) Restart FIJI

Run:

1) Open desired DIC .tif image (preferably one where the nuclei is not visible [see samples below]).

2) Once the image has opened, select the Morphological Segmentation plugin from *Plugins > MorphoLibJ > Segmentation > Morphological Segmentation*. A GUI interface will open for this plugin.

[The following instructions are for generic cell border images with reasonable contrast, but settings may vary.]

3) Under Input Image, set as a border image.

4) Under Watershed Segmentation, turn on Advanced Options, check Calculate Dams, and set Connectivity to 8.

5) To find the appropriate level of segmentation, set tolerance on the order of thousands and adjust accordingly. Lower tolerances result in more segmentation.

6) Under Results, adjust Display to personal preference. Overlaid Basins show colored segments superimposed over the original image, Overlaid Dams show segmentation lines superimposed over the original image, Catchment Basins show the colored segments, and Watershed Lines show only the segment lines.

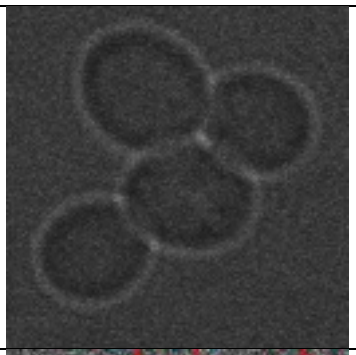
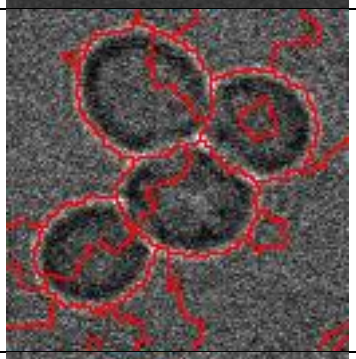
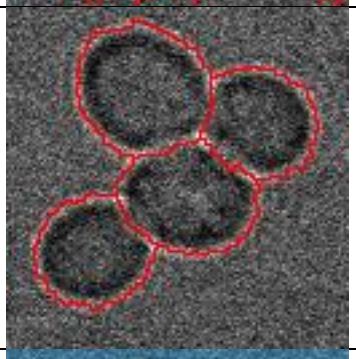
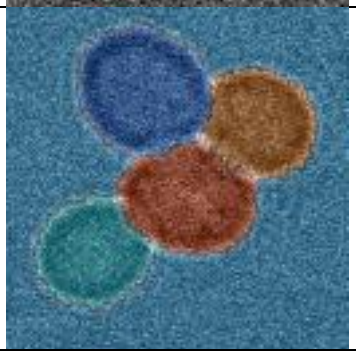
7) Click Create Image to output a segmented .tif image for saving and post-processing.

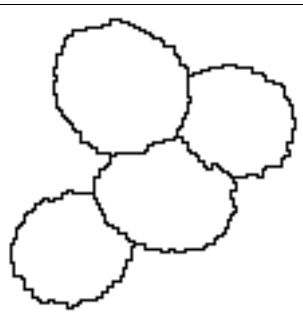
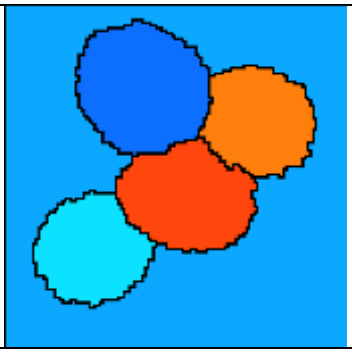
Advanced (Macros):

To automate the segmentation process, a complete macro example is shown below:

```
//Enter in filepath of .tif image
open("/FILEPATH.tif");

//Run plugin
run("Morphological Segmentation");
wait(1000);

//Run watershed segmentation
//Change tolerance level from default of tolerance=6200 to achieve appropriate results
//Lower numbers mean more segmentation and vice versa.
//Dams are calculated and are set at 8.
call("inra.ijpb.plugins.MorphologicalSegmentation.segment", "tolerance=6200",
"calculateDams=true", "connectivity=26");

//Option 1: Segmentation with just watershed lines
//call("inra.ijpb.plugins.MorphologicalSegmentation.setDisplayFormat", "Watershed lines");

//Option 2: Segmentation with the watershed lines superimposed over original image
//call("inra.ijpb.plugins.MorphologicalSegmentation.setDisplayFormat", "Overlaid dams");

//Option 3: Segmentation image with colored basins
//call("inra.ijpb.plugins.MorphologicalSegmentation.setDisplayFormat", "Catchment basins");

//Option 4: Segmentation image with colored basins superimposed over original image
//call("inra.ijpb.plugins.MorphologicalSegmentation.setDisplayFormat", "Overlaid basins");

//Create segmented image from selected option
call("inra.ijpb.plugins.MorphologicalSegmentation.createResultImage");
```

Examples:

DIC Image Input		
Overlaid Dams Output	Tolerance = 7000	
Overlaid Dams Output	Tolerance = 6200	
Overlaid Basins Output	Tolerance = 6200	

Watershed Lines Output	Tolerance = 6200	
Catchment Dams Basins	Tolerance = 6200	

References:

[1] https://imagej.net/MorphoLibJ
[2] https://imagej.net/Morphological_Segmentation
[3] https://imagej.net/Following_an_update_site#Add_update_sites
[4] https://github.com/ijpb/MorphoLibJ