NUCLEUS SEGMENTATION

AUTOMATE MULTICHANNEL FLUORESCENCE ANALYSIS

By

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Nucleus segmentation

Nucleus segmentation is a fundamental task in microscopy image analysis based on which multiple biological related analysis can be performed.

Cell or cell nuclei segmentation is typically the first critical step for biomedical microscopy image analysis. On the basis of accurate cell or cell nuclei segmentation, multiple biological or medical analysis can be performed subsequently, including cell type classification, particular cell counting, cell phenotype analysis etc., providing valuable diagnostic information for doctors and researchers. Although conventional image processing techniques are still employed for this time and labor consuming task, they often cannot achieve the optimized performance due to multiple reasons, such as limited capability of dealing with diverse images.

For automating these multichannel image analysis we are using ImageJ image progressing. Specifically we are using the Fiji app (image processing package - distribution of ImageJ2). We can build plugins which facilitate scientific image analysis.

For this project we have developed a plugin to automate the nucleus segmentation with provided image test dataset.

Details about the plugin are as follows;

Source: Github

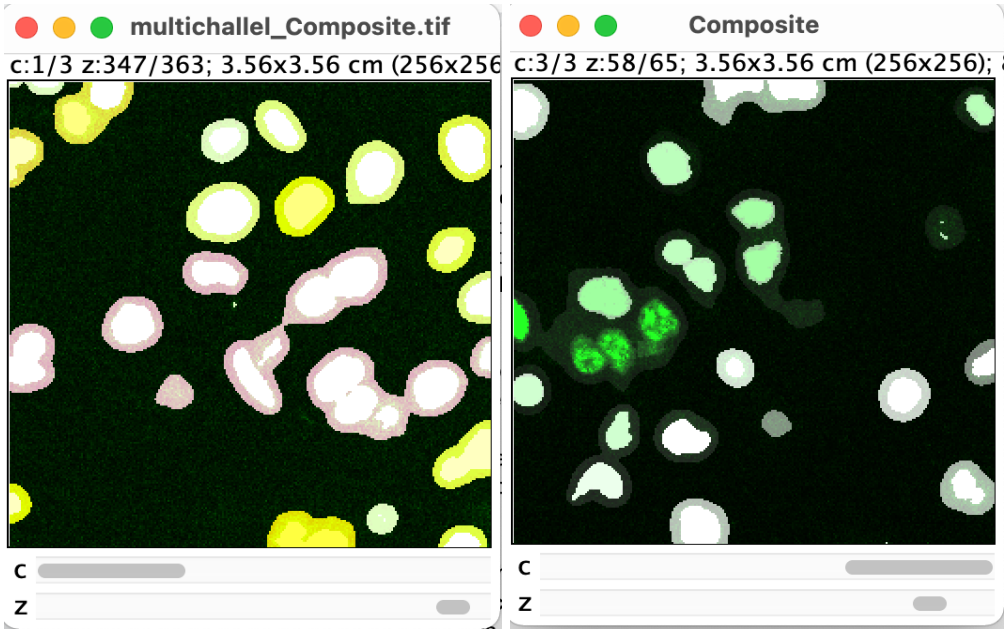
Installation:

- Clone the repository or download as the zip file
- The jar file will be available as well

Purpose:

- From this plugin we can get the statistics of the number of nucleus objects and the number of nucleolus present within the nucleus objects with various details about the volume, size, distance etc. And the final stat can be saved in csv format.

Results:



M_Nucleolus3DResultsMeasure3.csv

	Nb	Name	Label	Type	CX (pix)	CY (pix)	CZ (pix)	CX (unit)	CY (unit)	CZ (unit)
1	0	obj1-val1	1	0	40.201	25.003	27.155	13.306	8.276	8.988
2	1	obj2-val2	2	0	199.675	17.134	20.269	66.092	5.671	6.709
3	2	obj3-val3	3	0	182.281	15.605	0.000	60.335	5.165	0.000
4	3	obj4-val4	4	0	240.500	15.000	0.000	79.606	4.965	0.000
5	4	obj5-val5	5	0	192.571	19.857	0.000	63.741	6.573	0.000
6	5	obj6-val6	6	0	134.279	40.019	5.825	44.446	13.246	1.928
7	6	obj7-val7	7	0	240.846	67.308	0.000	79.720	22.279	0.000
8	7	obj8-val8	8	0	251.241	69.000	0.000	83.161	22.839	0.000
9	8	obj9-val9	9	0	247.985	83.744	0.000	82.083	27.719	0.000
10	9	obj10-val10	10	0	237.898	123.383	9.174	78.744	40.840	3.036
11	10	obj11-val11	11	0	29.599	120.711	0.000	9.797	39.955	0.000
12	11	obj12-val12	12	0	53.468	140.016	19.027	17.698	46.345	6.298
13	12	obj13-val13	13	0	136.072	181.666	15.442	45.040	60.132	5.111
14	13	obj14-val14	14	0	253.093	150.117	7.423	83.774	49.689	2.457
15	14	obj15-val15	15	0	74.745	202.927	22.061	24.741	67.169	7.302
16	15	obj16-val16	16	0	213.482	174.939	5.657	70.663	57.905	1.872
17	16	obj17-val17	17	0	169.616	176.294	4.044	56.143	58.353	1.339
18	17	obj18-val18	18	0	3.098	177.745	0.275	1.025	58.834	0.091
19	18	obj19-val19	19	0	186.190	207.513	9.231	61.629	68.687	3.055
20	19	obj20-val20	20	0	77.234	221.574	0.000	25.564	73.341	0.000
21	20	obj21-val21	21	0	65.503	241.345	5.037	21.681	79.885	1.667
22	21	obj22-val22	22	0	4.421	241.263	0.000	1.463	79.858	0.000
23	22	obj23-val23	23	0	162.667	253.632	8.194	53.843	83.952	2.712

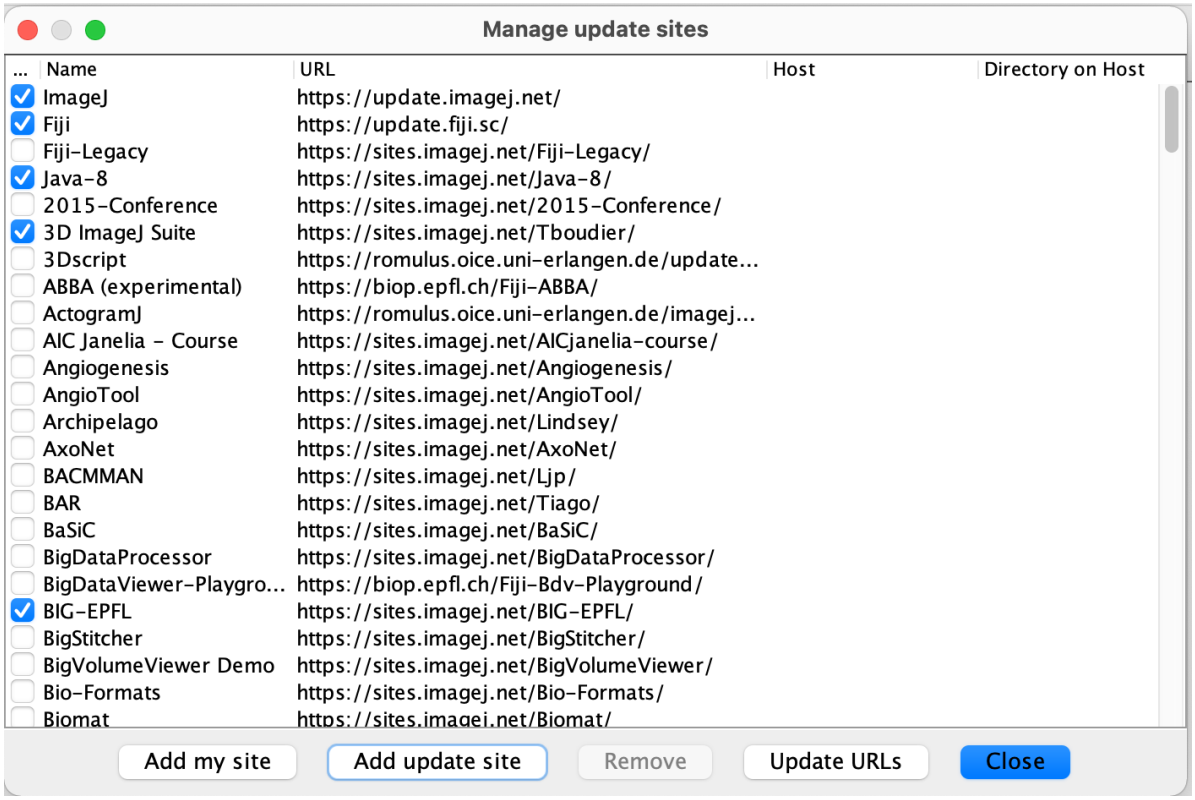
Run Nucleus segmentation plugin / macro

Prerequisites:

Download the latest Fiji Application with ImageJ2
https://imagej.net/software/fiji/downloads

Check this update sites:

Help> Update > Manage update sites >



How it works:

- We can use this project as plugin or macro
- As plugin we are packaging macro with in a jar file and running it using script service

Steps:

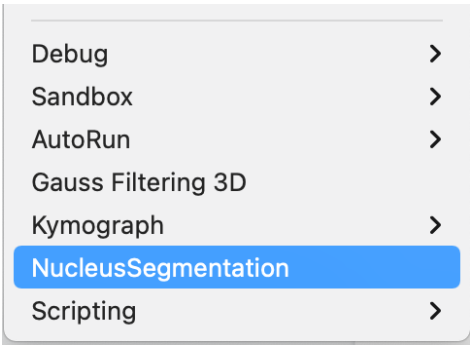
1. Select the image dataset
2. Input the the necessary parameters for segmentation
3. And analyze final composed image and statistics in the result
4. Overall segmented result are saved in csv format in following location;

   Downloads>"nucleus_segmentation_results" folder image filename

   Example: c.tif_nuclues_segmentaion_result2.csv

How to run with jar file:

- Download the jar file into the jars folder or plugin folder within your Fiji app
- Restart application and the plugin should be available in "Plugin tab" Navigate > Plugins and scroll down "NucleusSegmentation" should be there



Then we can select the image dataset and click on "**NucleusSegmentation**" . It should start the image processing with necessary input dialogs and logs.

How to run as macro:

- Open the data in your app
- Navigate to Plugins → Macros → Run
- Navigate to the macro file in the repository and run with your chosen parameters
  - In the current repository the macro is located here:
    - /bin/src/main/resources/nucleus_seg_macro.ijm

How to run as plugin with the downloaded repository:

- Import the project into Eclipse
- Add a run configuration set up like this:
    - The base directory housing the plugin repository
    - Add a parameter with the following values
        - Name: *scijava.app.directory*
        - Value: /path/to/ImageJ.app
- Now run the project, there should now be a jar file in the /target directory once the build completes
- Move this jar file to your ImageJ or Fiji app, you can do this by running the following:
    - mvn -Dscijava.app.directory=/path/to/ImageJ.app/
- Refresh your app and then you should see the plugin in the Plugin menu!

The plugin performs the following steps in background :

- Finds the nucleus objects in the data
    - run("Duplicate...", "title=imgDUP duplicate");
    - run("Gaussian Blur...", "sigma=GaussianBlur stack");
    - run("Subtract Background...", "rolling=SubtractBackground stack");
    - run("HiLo");
    - run("Subtract...", "value=Subtract stack");
    - run("3D Simple Segmentation", "low_threshold=SimpleSegmentationThreshold min_size=SimpleSegmentationMin max_size=SimpleSegmentationMax");
    - run("3-3-2 RGB");
- Segments all nucleoli per each nucleus
    - resetThreshold;
    - setOption(“BlackBackground”, true);
    - selectWindow(nucleus_object_IMG_tit);
    - run(“Duplicate…”, “title=tempDUP_NUCL duplicate”);
    - setThreshold(n, n);
    - run(“Convert to Mask”, “method=Default background=Dark black”);
- Segments multiple times and merges the channels found
- Then the statistics are output and saved into a csv format for the user

Features in the plugin/macro

- Made platform independent for read and write file access (tested in windows / mac os)
- Made possible to package macro into jar to run as plugin
- Proper github code to further updates and analysis according to need
- Easier to test with marco and build it as jar file to run as plugin
- Saves .csv results in the download folder for further analysis