Nucleus Segmentation Plugin

Steps done for segmentation

1. Find Nucleus objects
2. Find Nucleoli per nucleus
3. Do segmentation multiple couple of times and merge those channels and display composed image
4. Save the 3D Measure statistics in csv format

1. Find Nucleus objects

```
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");
```

2. Find Nucleoli per nucleus

- Find nucleolus_per_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");
```

- Find 3D NUCLEOLUS

How we made macro to run as plugin

- Read the file as a stream

```
java.io.InputStream in = getClass().getResourceAsStream("/nucleus_seg_macro.ijm");
```

```
BufferedReader reader = new BufferedReader(new InputStreamReader(in));
```

- Save it as a content

```
macro_content = ReadBigStringIn(reader);
```

- And finally run the content as macro using “***scriptService***”

```
scriptService.run(".ijm", macro_content, true, image).get();
```

How we made macro to run as plugin

- Write a plugin for Fiji
- Added the necessary macro in the resource folder
- Plugin cannot read the macro directly as a content from jar file
- Locate the macro file included with the help of “**getClassLoader”**

```
java.net.URL resource = getClass().getClassLoader().getResource("nucleus_seg_macro.ijm");
```

How we made platform independent

```
// Get path to temp directory
tmp = getDirectory("temp");
if (tmp=="")
    exit("No temp directory available");
// Create a directory in temp
myDir = tmp+"my-test-dir"+File.separator;
File.makeDirectory(myDir);
if (!File.exists(myDir))
    exit("Unable to create directory");
```

Setup Prerequisites

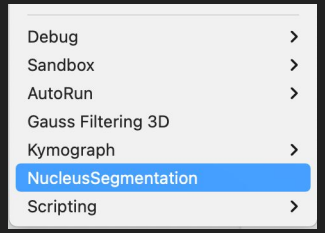
Prerequisites to run the project : Download the latest Fiji Application

- Update the fiji application
- Also in ImageJ updater

Help> Update > Manage update sites >

Check these sites

- ImageJ
- Fiji
- Java-8
- 3D ImageJ Suite
- Big-EPFL
- ResultsToExcel

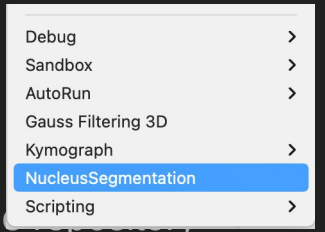


Fiji download link:

https://imagej.net/software/fiji/downloads

Setup

- Use .jar (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”
- Use .ijm (marcro) file
  - Download the macro file
  - Navigate to Plugins → Macros → Run and selected the downloaded file
- Build the plugin using repository code
  - Build the project following the necessary steps mentioned in Readme in code repository https://github.com/RajanKent/nucleus-segmentation-plugin



How it works

We can run this project as plugin / macro

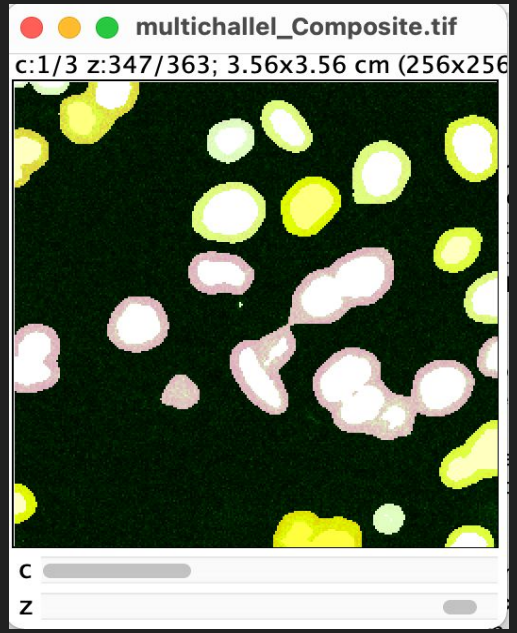
- Select the image dataset
- Input the the necessary parameters for segmentation
- And analyze final composed image and statistics in the result

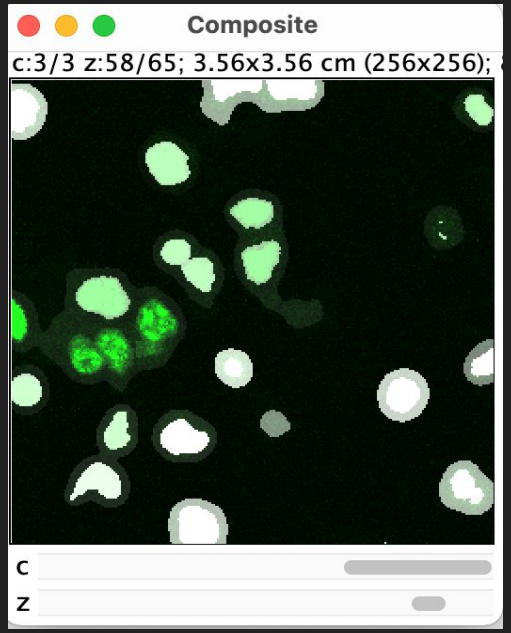
Overall segmented result are saved in csv format

Downloads>”nucleus_segmentation_results” folder image filename

Example: c.tif_nuclues_segmentaion_result2.csv

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

Nucleus Segmentation Plugin
Demo