ADVANCED BIOIMAGING FACILITY

ABIF workshop
Introduction to writing macros in Fiji

with Joel Ryan

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https://github.com/ABIF-McGill/Intro_to_Fiji_Macros

Introduction to writing macros in Fiji

- Goal:
  - Learn a general workflow to write macros in Fiji in order to help:
    - Process images
    - Extract data from images

- You will need to download:
  - **Fiji**
    - **fiji.sc**

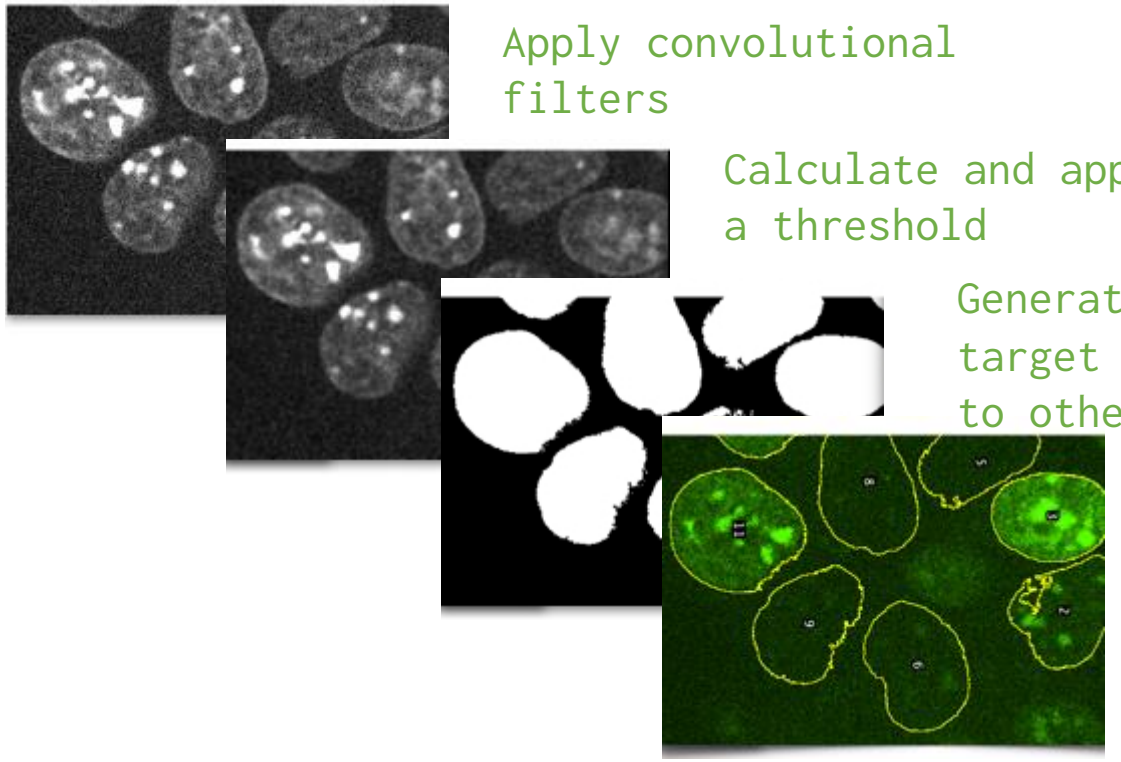
- Sample image data:
  - **https://github.com/ABIF-McGill/Intro_to_Fiji_Macros**

Why write macros/scripts?

- Streamline processing and data extraction
- Help develop more robust and reliable assays

--> Relieve the bottleneck of tedious analysis

Open image, adjust contrast, duplicate



Apply convolutional filters

Calculate and apply a threshold

Generate ROIs, select target image, apply ROIs to other channel(s)

Measure intensity data in each ROI

Repeat this process on
10-30 images x 3 replicates x n genotypes/conditions…

Today’s workshop

- Walk through writing three macros
  1. Make a composite image
  2. Apply filters, threshold and measure intensities
  3. Manual analysis template / quality control

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> IJ1 macro language

- Targeted for image processing/analysis
- Written commands underlying point-and-click ImageJ/Fiji
- Great intro to programming for bio people

https://github.com/ABIF-McGill/Intro_to_Fiji_Macros

Today: we are a big group!

- There are lots of participants today!

- To keep things moving:
  - Please post questions in the Zoom chat
  - We'll try our best to answer as many questions as we can

- If things aren't working:
  - Take note of the error message and line
  - Try copy-pasting the script from the github repository…

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Challenges for you beyond this workshop

- Figure out the processing and analysis needed for your imaging experiments:
  - Conceptually, what is the question?
  - -> practically:
    - what quantitative parameters do you need to extract from your images
    - which functions do you need to run to get that data
    - do you need to retool your image acquisition settings…?
- … then find good ways to plot and present your data!

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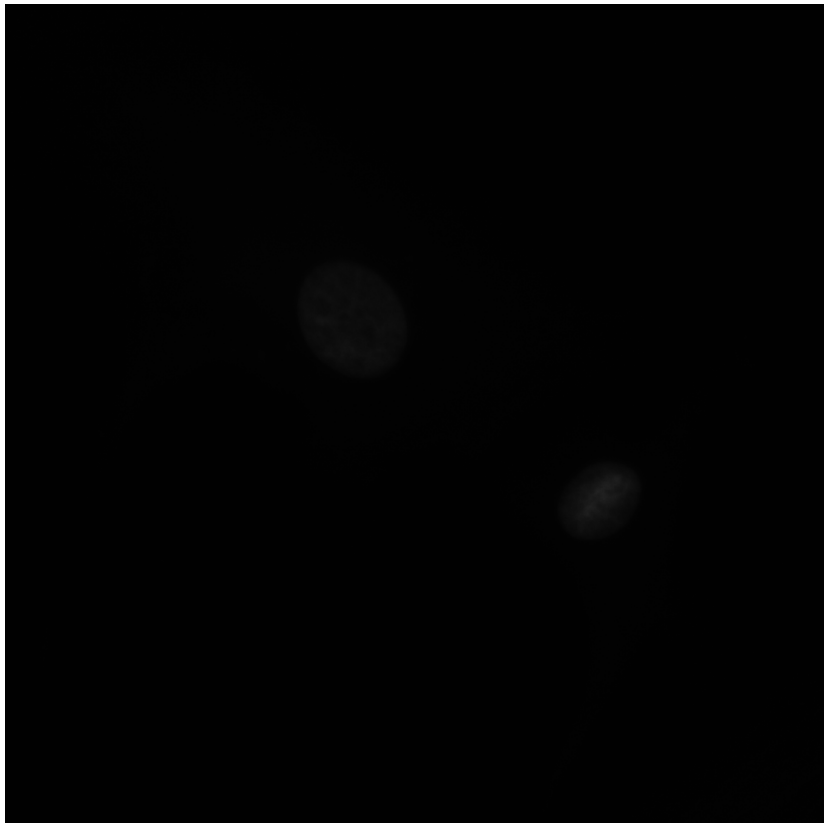
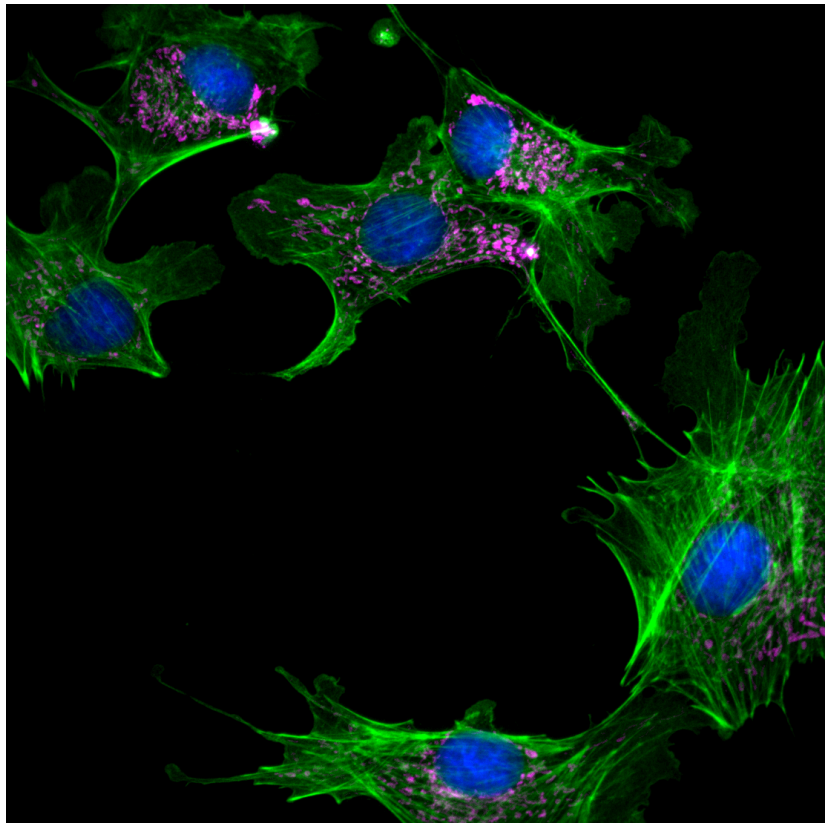
Exercise 1 – raw vs composite RGB images



demo_DAPI_Phalloidin_Mitotracker_001.tif

Raw image, drag-and-drop in powerpoint.



demo_DAPI_Phalloidin_Mitotracker_001.tif (RGB).tif

Composite RGB image generated with Exercise 1 macro, drag-and-drop in powerpoint.

for loop

```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

for loop

Start value

End value

Increment

```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

for loop

Start value

End value

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```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

first, “build a sequence of integers going from the Start Value, to the End value, with an increment of 1”

for loop

Start value End value Increment

```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

first, “build a sequence of integers going from the Start Value, to the End value, with an increment of 1”

---in the case of numROI being 5:

0, 1, 2, 3, 4

for loop

Start value End value Increment

```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

first, “build a sequence of integers going from the Start Value, to the End value, with an increment of 1”

---in the case of numROI being 5:

0, 1, 2, 3, 4,

“Run the { content of the loop } replacing **i** with the first integer of the sequence.

Then run the { content of the loop } replacing i with the second integer in the sequence… “ and so on…

for loop

Start value End value Increment

```
for (i = 0; i < numROI; i++) {

    roiManager("Select", i);
    Stack.setChannel(2);
    run("Measure");

}
```

```
i = 0
roiManager("Select", i);
Stack.setChannel(2);
run("Measure");

i = 1
roiManager("Select", i);
Stack.setChannel(2);
run("Measure");

i = 2
roiManager("Select", i);
Stack.setChannel(2);
run("Measure");

i = 3
roiManager("Select", i);
Stack.setChannel(2);
run("Measure");

...
```