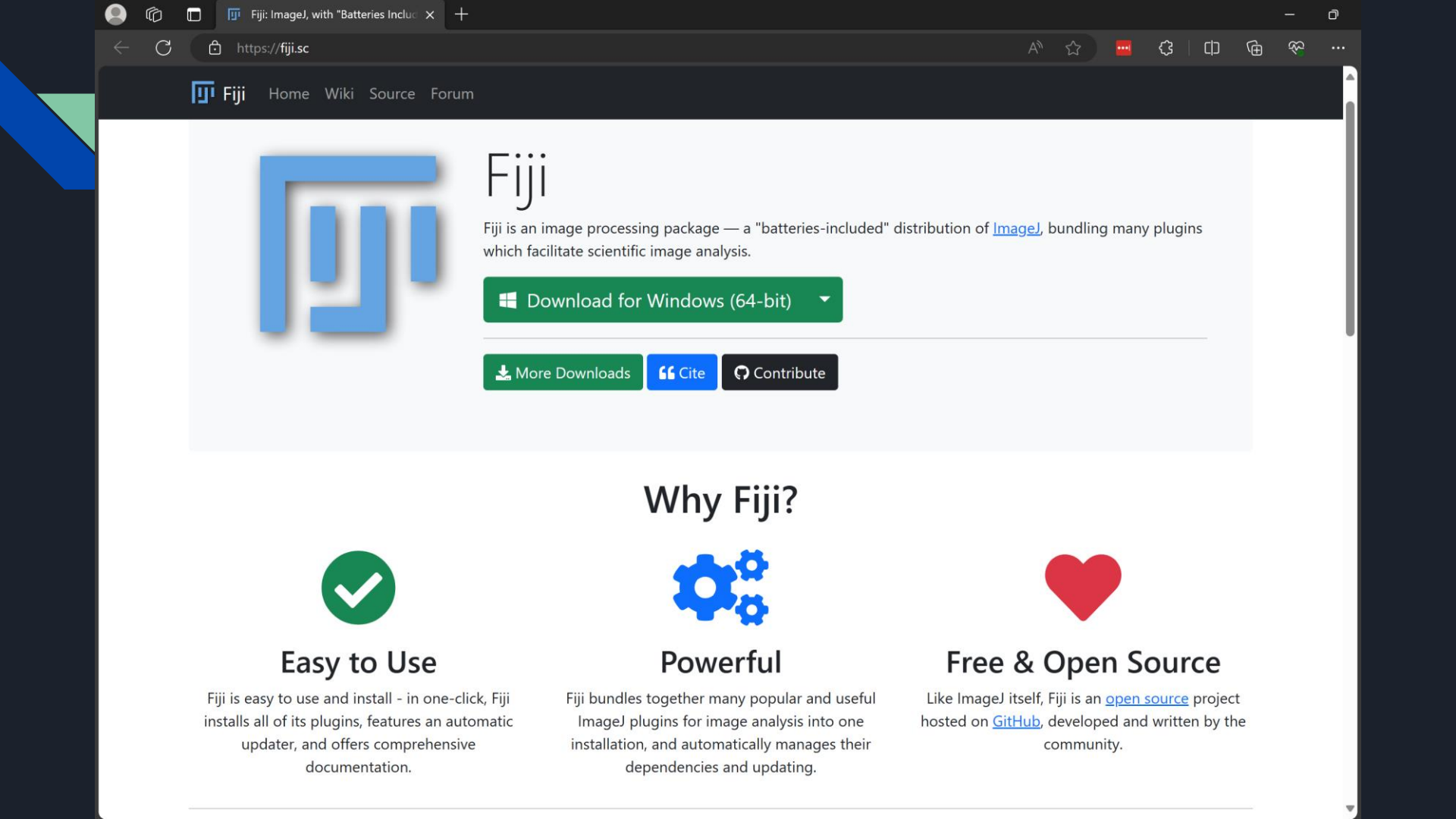
Introduction to ImageJ Macros

Justin Savage

Fiji Is Just ImageJ

- Available at fiji.sc


Fiji: ImageJ, with "Batteries Includ
https://fiji.sc
Fiji
Home
Wiki
Source
Forum
Fiji
Fiji is an image processing package — a "batteries-included" distribution of ImageJ, bundling many plugins which facilitate scientific image analysis.
Download for Windows (64-bit)
More Downloads
Cite
Contribute
Why Fiji?
Easy to Use
Fiji is easy to use and install - in one-click, Fiji installs all of its plugins, features an automatic updater, and offers comprehensive documentation.
Powerful
Fiji bundles together many popular and useful ImageJ plugins for image analysis into one installation, and automatically manages their dependencies and updating.
Free & Open Source
Like ImageJ itself, Fiji is an open source project hosted on GitHub, developed and written by the community.

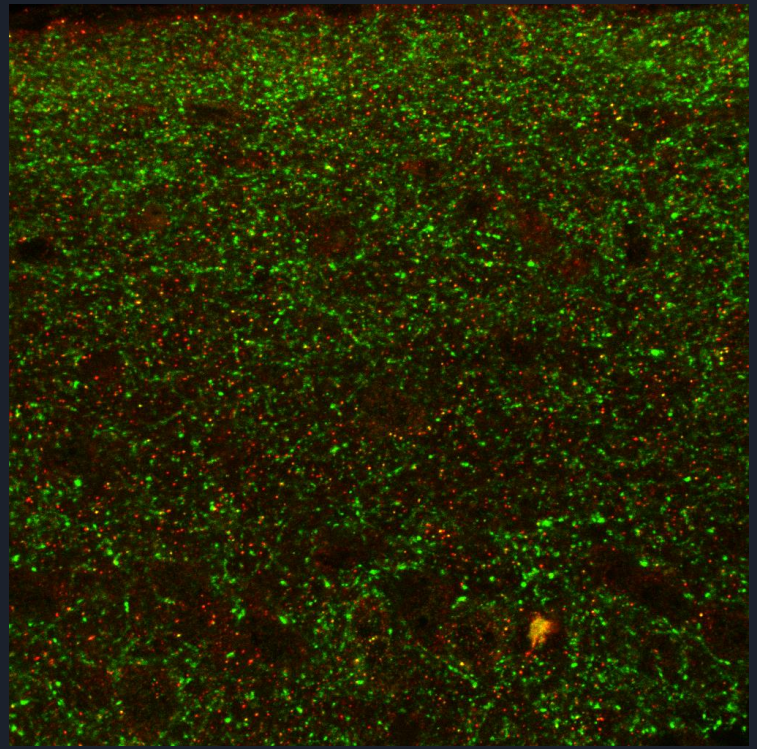
What can you do with ImageJ Macros?

- Count objects in an image
- Measure intensity values of for many cells
- Write relevant measurements to a table/CSV file
- Quantify the morphology of a cell
- Convert image files from one type to another
- And many more!

CS step one: Solving the problem on paper

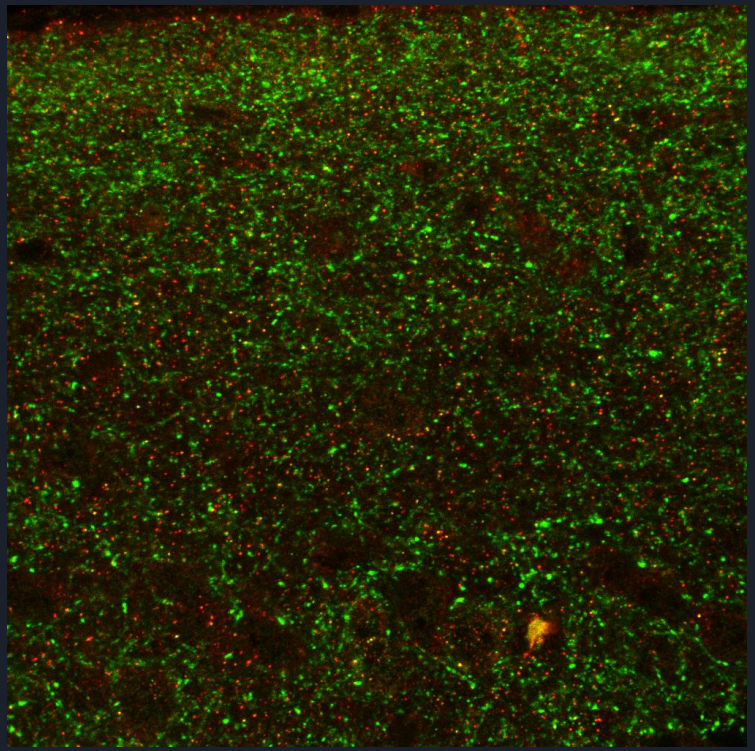
- Computers can’t do something you don’t know how to do
- Computers are for increasing scale and efficiency

Problem: counting PSD95 puncta

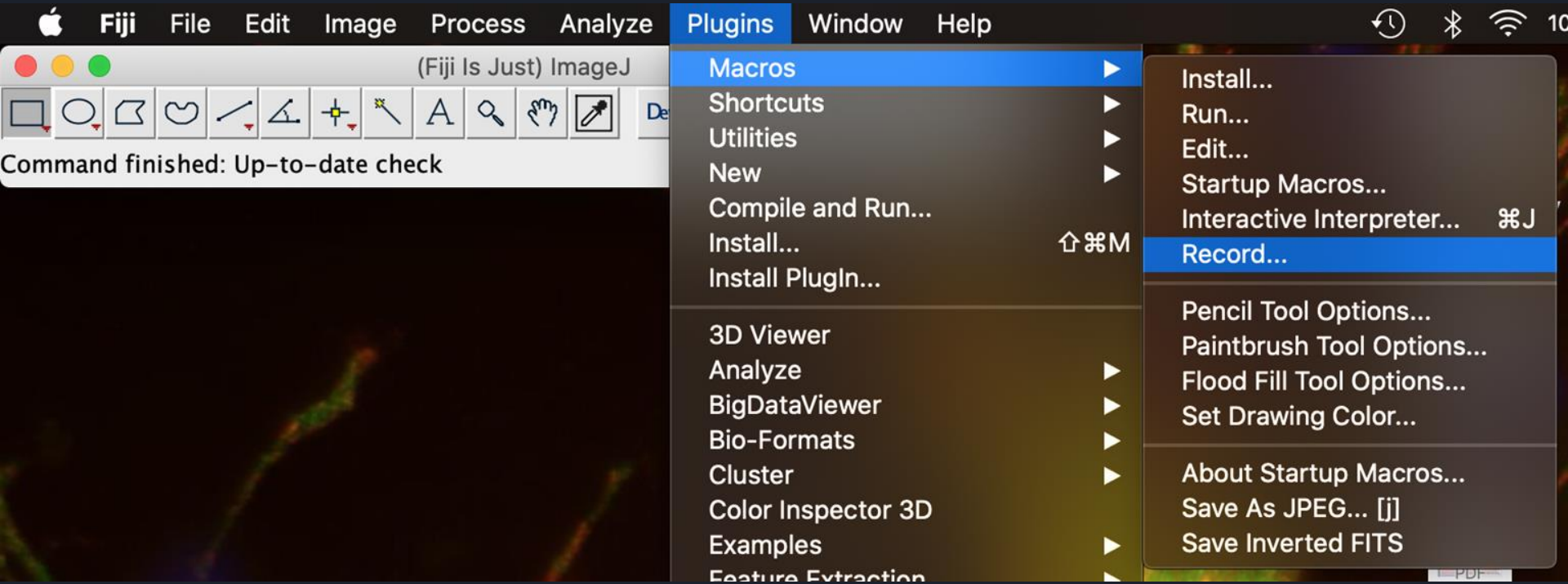


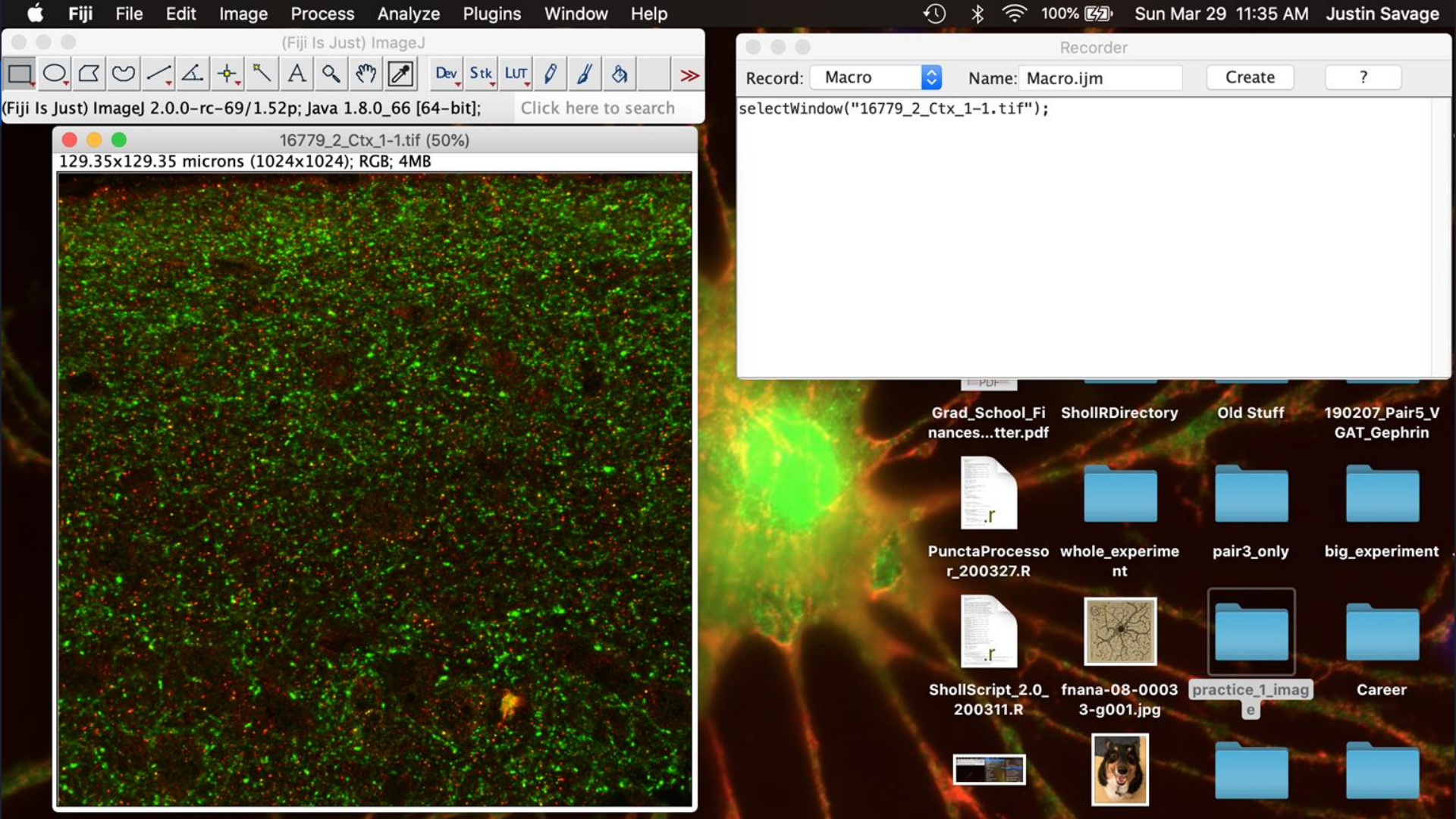
Puncta analysis on paper

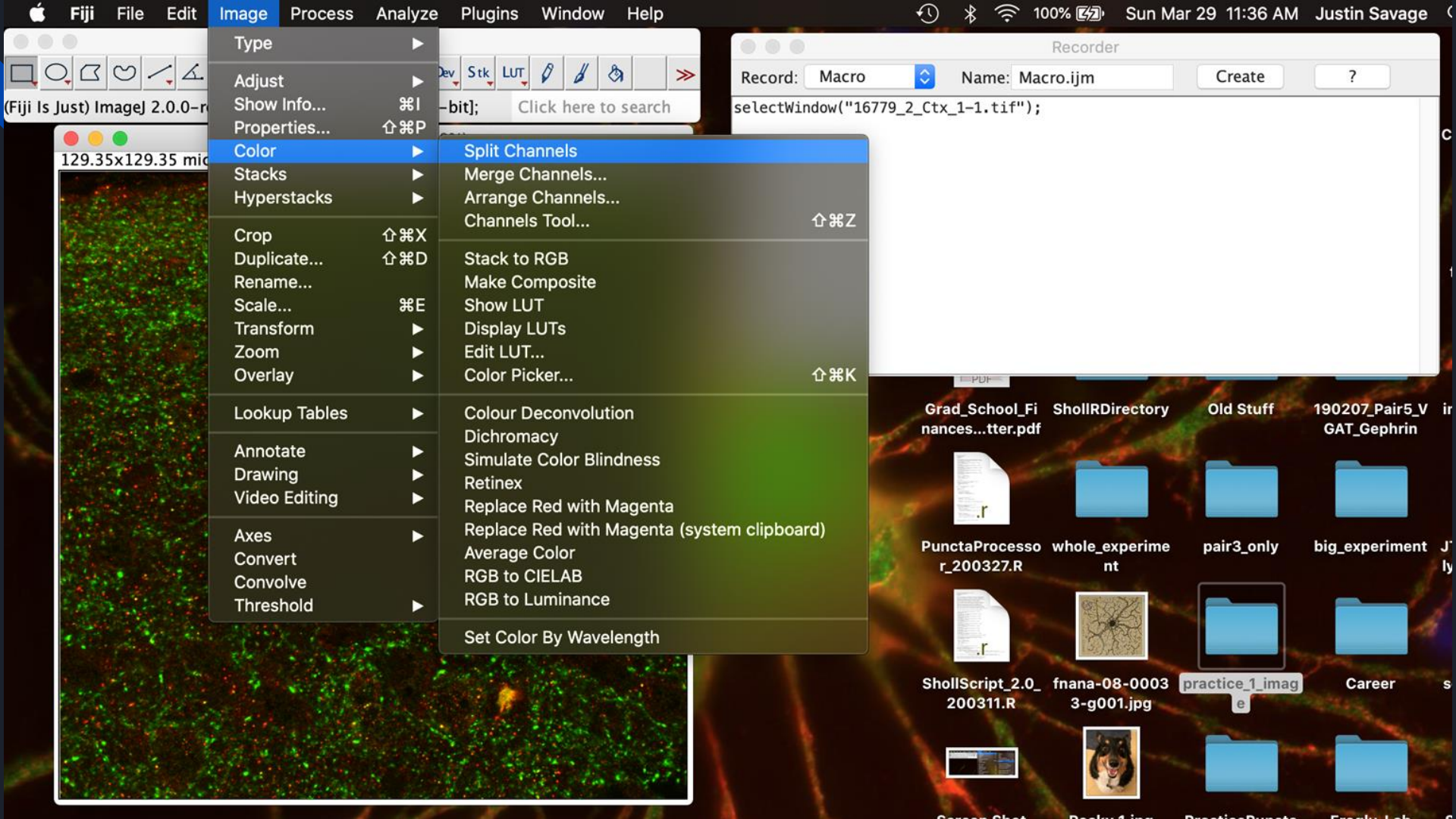
1) Split channels
2) Subtract background
3) Threshold image
4) Analyze particles
5) Save the results

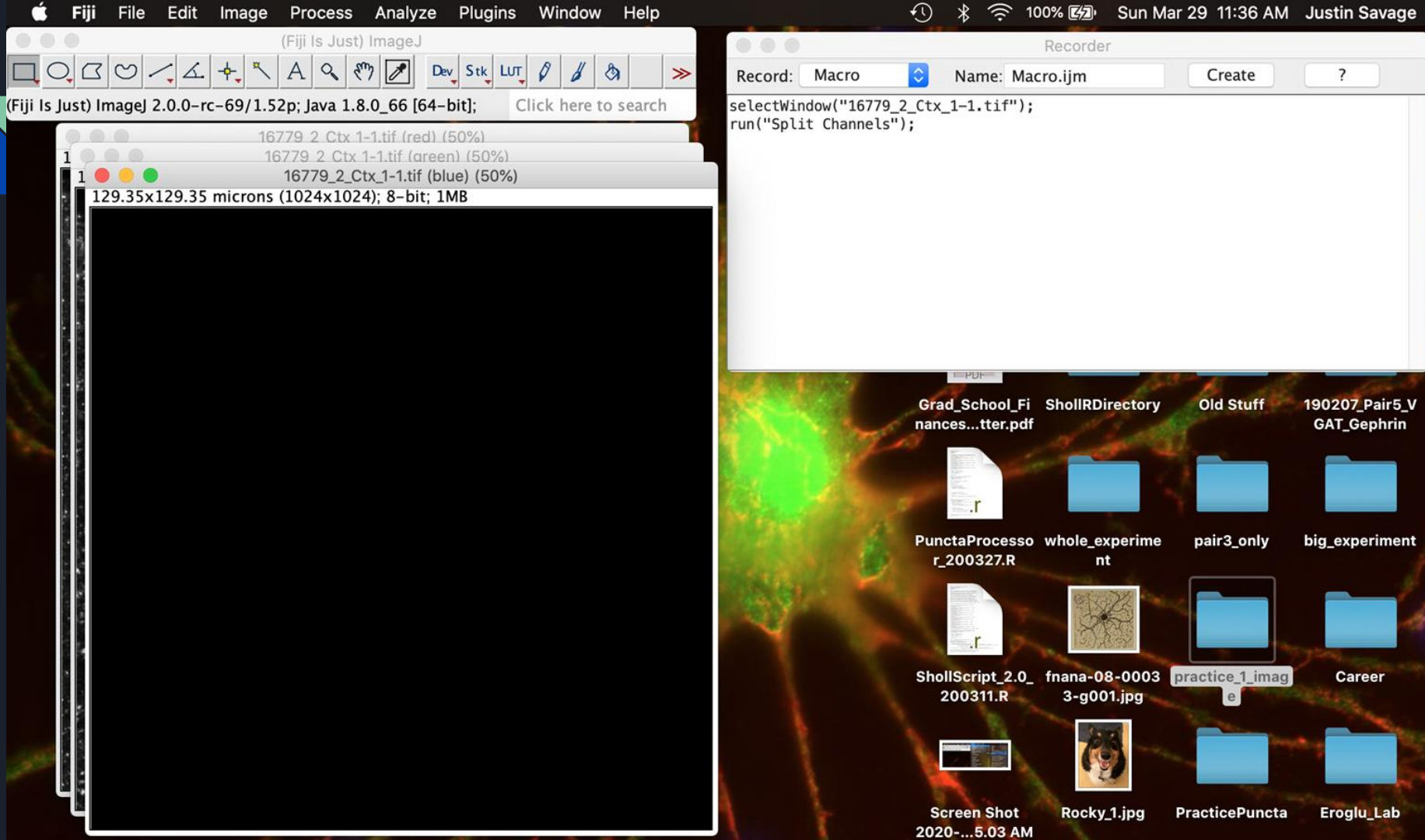


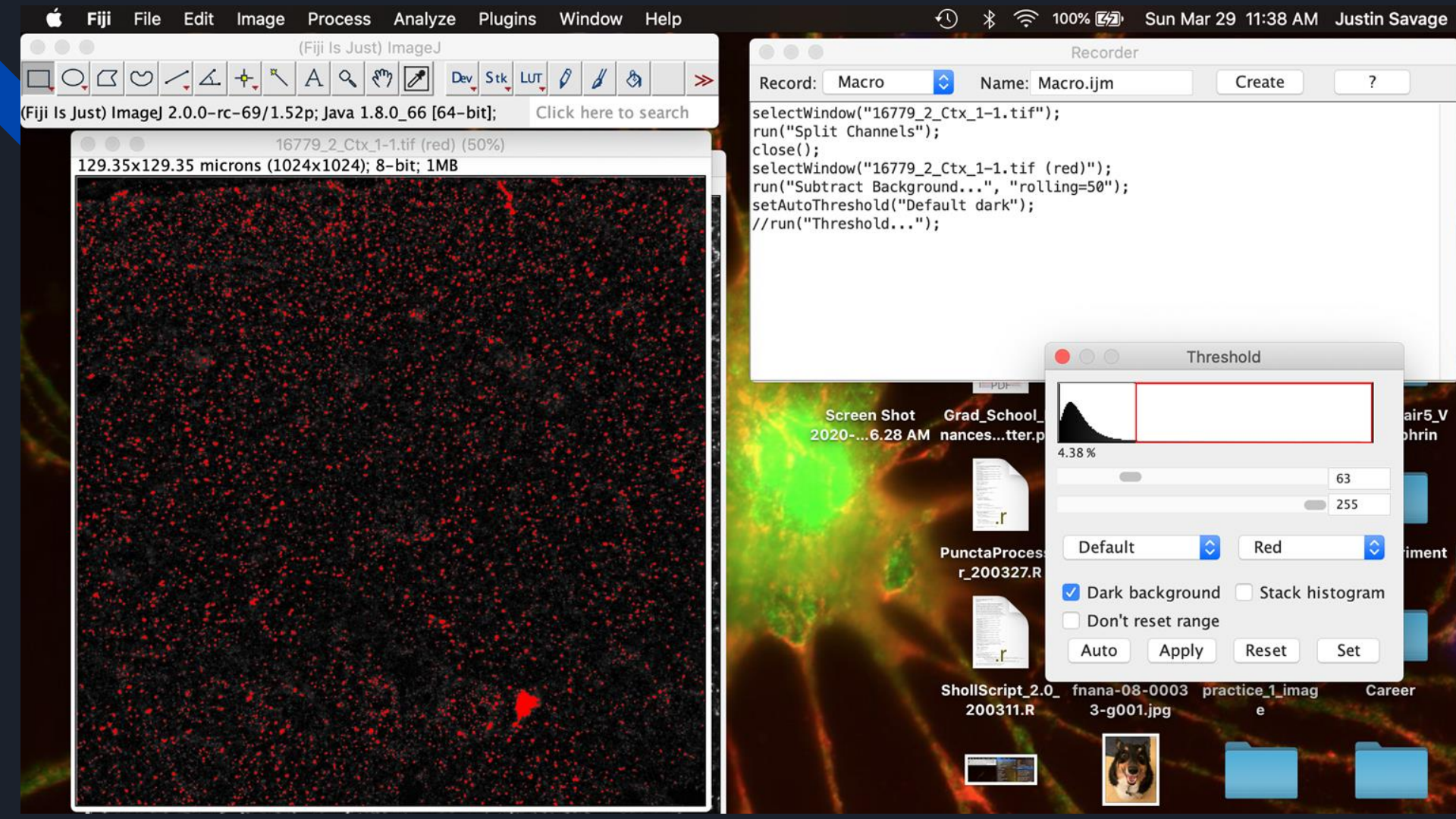
Macro recorder: a good place to start




Fiji File Edit Image Process Analyze Plugins Window Help
100% Sun Mar 29 11:35 AM Justin Savage
(Fiji Is Just) ImageJ
Dev Stk LUT
(Fiji Is Just) ImageJ 2.0.0-rc-69/1.52p; Java 1.8.0_66 [64-bit];
Click here to search
16779_2_Ctx_1-1.tif (50%)
129.35x129.35 microns (1024x1024); RGB; 4MB
Recorder
Record: Macro
Name: Macro.ijm
Create
?
selectWindow("16779_2_Ctx_1-1.tif");
Grad_School_Finances...tter.pdf
ShollRDirectory
Old Stuff
190207_Pair5_VGAT_Gephrin
PunctaProcessor_200327.R
whole_experiment
pair3_only
big_experiment
ShollScript_2.0_200311.R
fnana-08-00033-g001.jpg
practice_1_image
Career


Fiji File Edit Image Process Analyze Plugins Window Help
100% Sun Mar 29 11:36 AM Justin Savage
Type
Adjust
Show Info... ⌘I
Properties... ⇧⌘P
Color
Stacks
Hyperstacks
Crop ⇧⌘X
Duplicate... ⇧⌘D
Rename...
Scale... ⌘E
Transform
Zoom
Overlay
Lookup Tables
Annotate
Drawing
Video Editing
Axes
Convert
Convolve
Threshold
Split Channels
Merge Channels...
Arrange Channels...
Channels Tool... ⇧⌘Z
Stack to RGB
Make Composite
Show LUT
Display LUTs
Edit LUT...
Color Picker... ⇧⌘K
Colour Deconvolution
Dichromacy
Simulate Color Blindness
Retinex
Replace Red with Magenta
Replace Red with Magenta (system clipboard)
Average Color
RGB to CIELAB
RGB to Luminance
Set Color By Wavelength
Recorder
Record: Macro
Name: Macro.ijm
Create
?
selectWindow("16779_2_Ctx_1-1.tif");
Click here to search
Grad_School_Finances...tter.pdf
ShollRDirectory
Old Stuff
190207_Pair5_VGAT_Gephrin
PunctaProcessor_200327.R
whole_experiment
pair3_only
big_experiment
ShollScript_2.0_200311.R
fnana-08-00033-g001.jpg
practice_1_image
Career


Fiji File Edit Image Process Analyze Plugins Window Help
100% Sun Mar 29 11:36 AM Justin Savage
(Fiji Is Just) ImageJ
(Fiji Is Just) ImageJ 2.0.0-rc-69/1.52p; Java 1.8.0_66 [64-bit];
Click here to search
16779_2_Ctx_1-1.tif (red) (50%)
16779_2_Ctx_1-1.tif (green) (50%)
16779_2_Ctx_1-1.tif (blue) (50%)
129.35x129.35 microns (1024x1024); 8-bit; 1MB
Recorder
Record: Macro
Name: Macro.ijm
Create
?
selectWindow("16779_2_Ctx_1-1.tif");
run("Split Channels");
Grad_School_Finances...tter.pdf
ShollRDirectory
Old Stuff
190207_Pair5_VGAT_Gephrin
PunctaProcessor_200327.R
whole_experiment
pair3_only
big_experiment
ShollScript_2.0_200311.R
fnana-08-00033-g001.jpg
practice_1_image
Career
Screen Shot 2020-...5.03 AM
Rocky_1.jpg
PracticePuncta
Eroglu_Lab


Fiji File Edit Image Process Analyze Plugins Window Help
100% Sun Mar 29 11:38 AM Justin Savage
(Fiji Is Just) ImageJ
Dev Stk LUT
(Fiji Is Just) ImageJ 2.0.0-rc-69/1.52p; Java 1.8.0_66 [64-bit];
Click here to search
16779_2_Ctx_1-1.tif (red) (50%)
129.35x129.35 microns (1024x1024); 8-bit; 1MB
Recorder
Record: Macro
Name: Macro.ijm
Create
?
selectWindow("16779_2_Ctx_1-1.tif");
run("Split Channels");
close();
selectWindow("16779_2_Ctx_1-1.tif (red)");
run("Subtract Background...", "rolling=50");
setAutoThreshold("Default dark");
//run("Threshold...");
Threshold
4.38 %
63
255
Default
Red
Dark background
Stack histogram
Don't reset range
Auto
Apply
Reset
Set
Screen Shot 2020-...6.28 AM
Grad_School_nances...tter.p
PunctaProcessor_200327.R
ShollScript_2.0_200311.R
fnana-08-00033-g001.jpg
practice_1_image
Career

TextEdit File Edit Format View Window Help 100% Sun Mar 29 11:41 AM Justin Savage

(Fiji Is Just) ImageJ

(Fiji Is Just) ImageJ 2.0.0-rc-69/1.52p; Java 1.8.0_66 [64-bit];

Click here to search

16779_2_Ctx_1-1.tif (red) (50%)

129.35x129.35 microns (1024x1024);

Slice	Count	Total A
16779_2_Ctx_1-1.tif (red)	3527	657.78

Recorder

Record: Macro Name: Macro.ijm Create ?

```
selectWindow("16779_2_Ctx_1-1.tif");
run("Split Channels");
close();
selectWindow("16779_2_Ctx_1-1.tif (red)");
run("Subtract Background...", "rolling=50");
setAutoThreshold("Default dark");
//run("Threshold...");
run("Select All");
run("Analyze Particles...", "size=4-Infinity pixel display exclude clear
summarize add");
saveAs("Results", "/Users/Justin/Desktop/Results.csv");
```

	Area	Mean									
1	0.064	80.500	23.798	66	66	116	73.769	0.253	5.138	322	1
2	0.112	96.571	28.930	64	64	148	102.127	0.370	10.786	676	1
3	0.064	83.750	19.294	70	70	112	123.033	0.316	5.345	335	1
4	0.957	120.283	49.591	94	64	229	98.462	0.842	115.155	7217	1
5	0.638	115.250	53.560	67	63	223	64.106	0.891	73.557	4610	1
6	0.064	67.500	5.802	63	63	76	67.043	0.474	4.308	270	1
7	0.096	95.167	17.023	95	81	128	65.643	0.716	9.111	571	1
8	0.351	108.682	40.551	77	69	222	69.044	0.804	38.151	2391	1
9	0.223	148.071	62.776	68	68	218	73.896	0.731	33.077	2073	1
10	0.319	151.750	60.392	235	68	235	93.247	0.808	48.427	3035	1
11	0.191	137.167	69.975	66	63	234	108.528	0.789	26.264	1646	1
12	3.558	105.466	37.955	72	63	225	88.176	2.967	375.270	23519	1
13	0.096	77.833	16.461	64	64	109	90.759	0.758	7.451	467	1

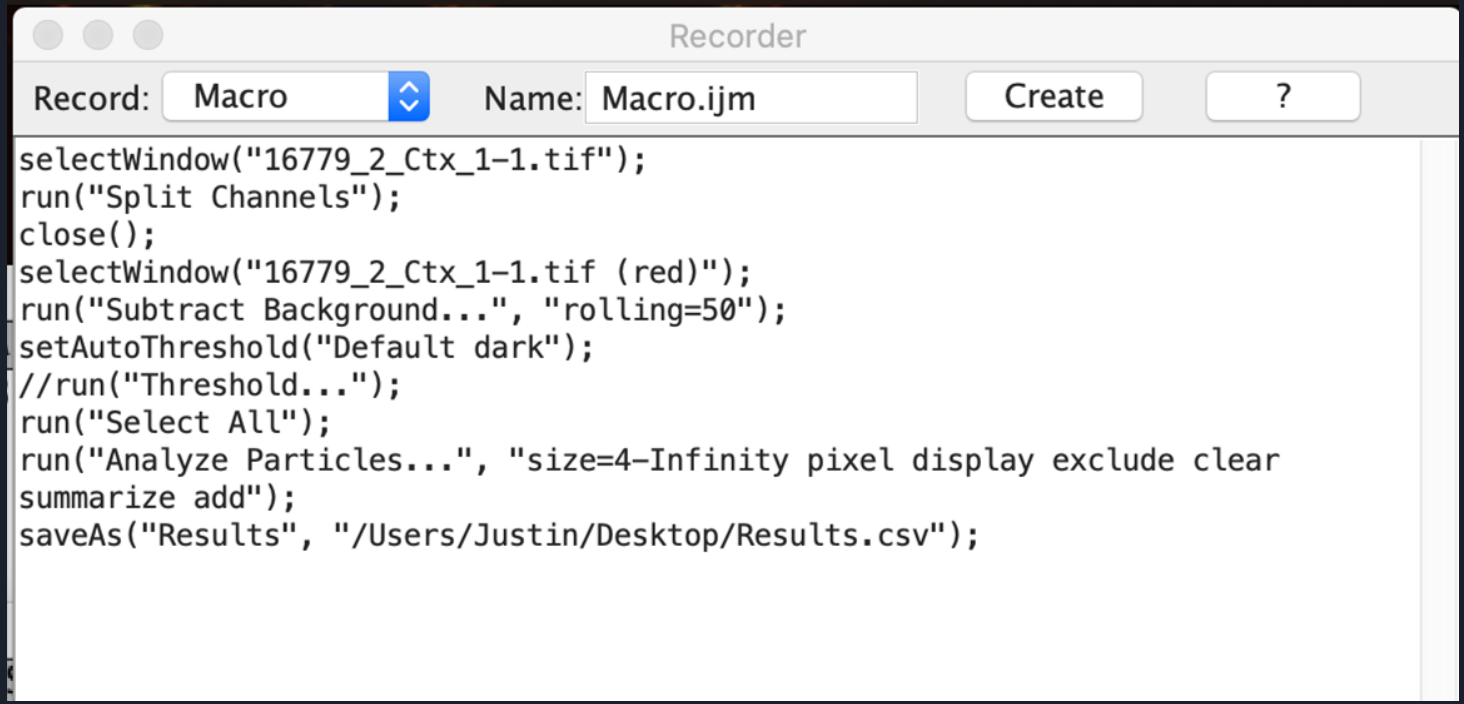
2020-...0.02 AM 200311.R 3-g001.jpg

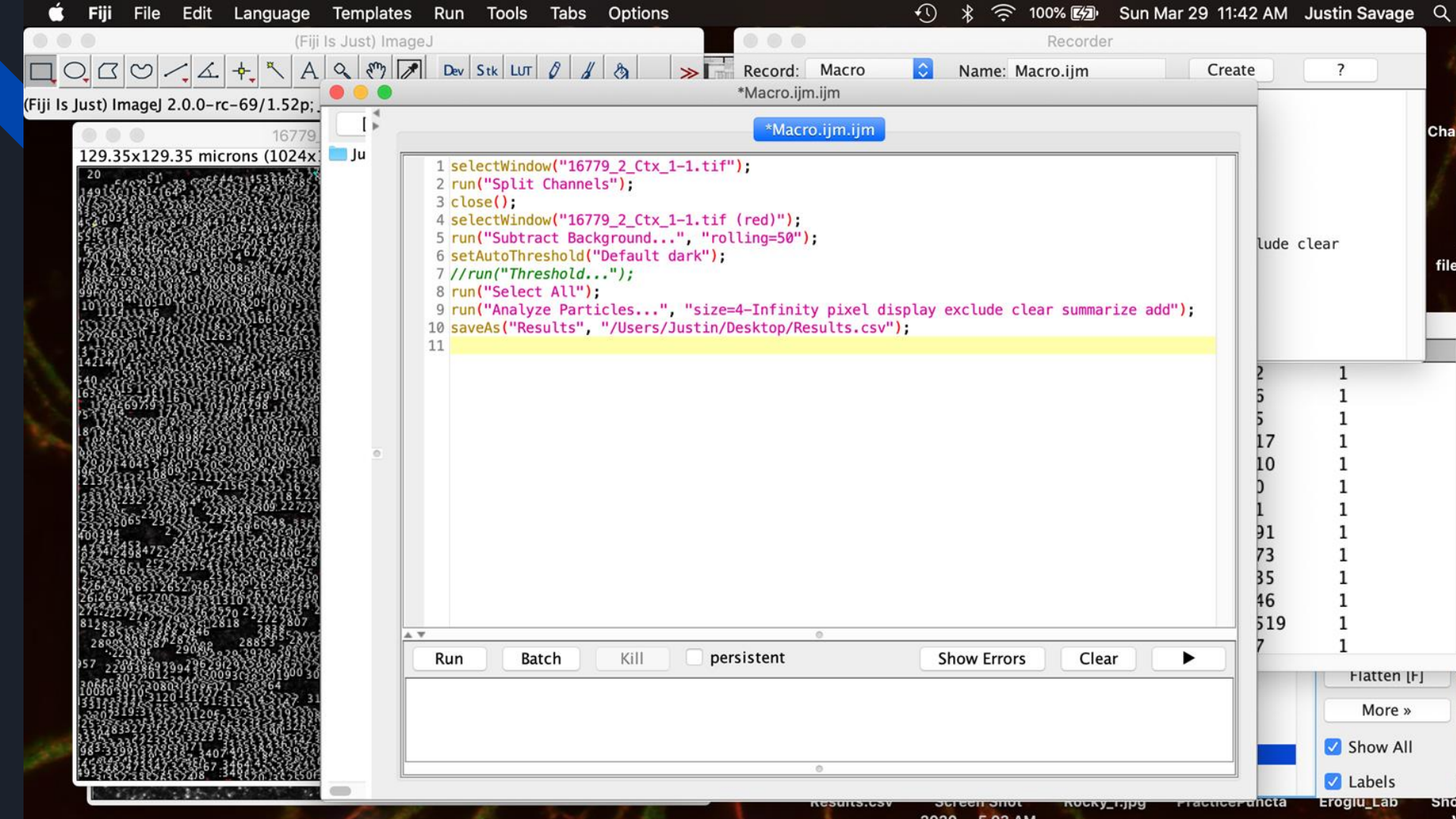
Results.csv Screen Shot 2020-...5.03 AM Rocky_1.jpg

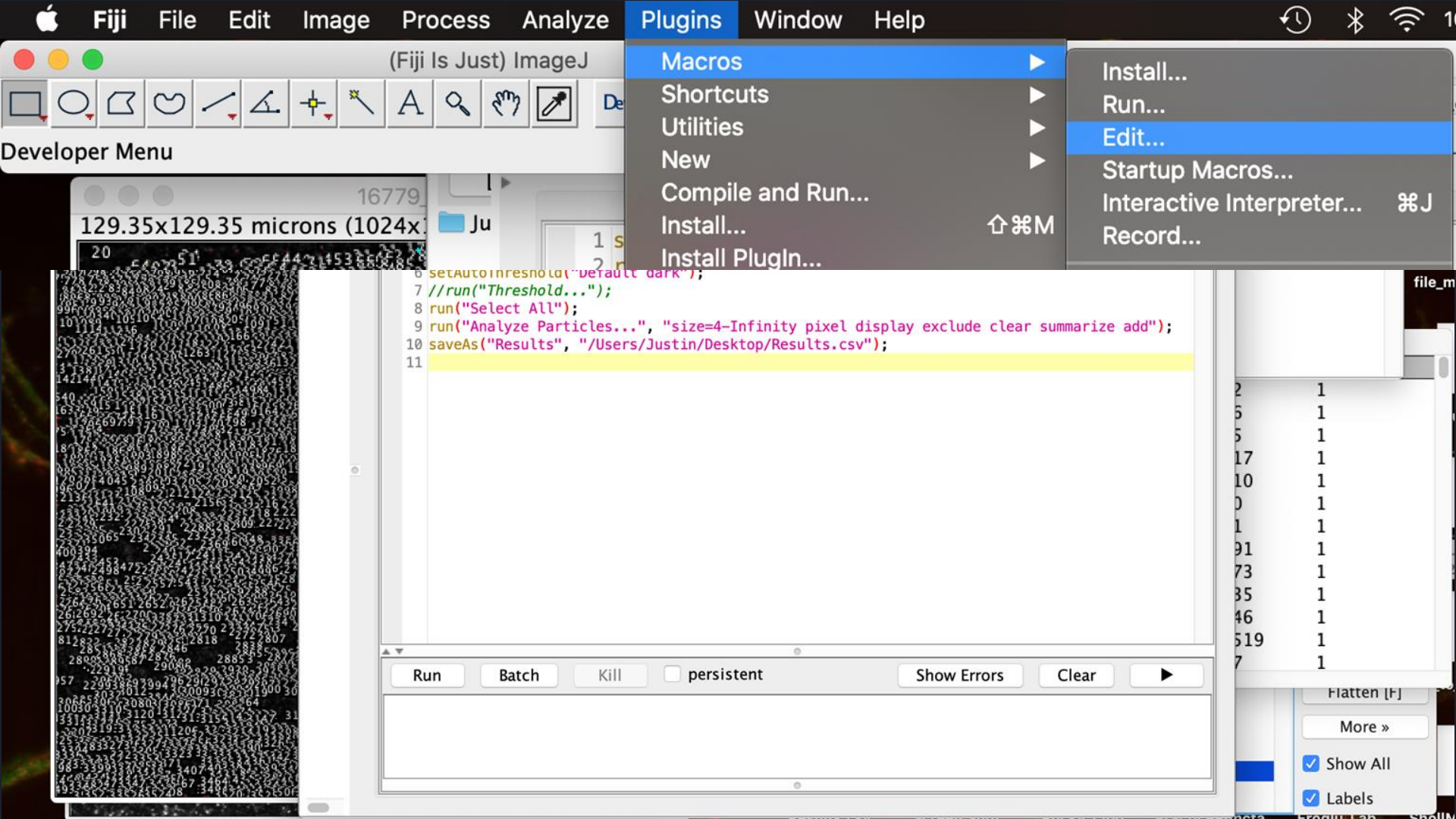
0013-0006
0014-0006
0015-0014
0016-0009
0017-0008
0018-0011

Flatten [F]
More »
Show All
Labels

PracticePuncta Eroglu_Lab


Recorder
Record: Macro
Name: Macro.ijm
Create
?
selectWindow("16779_2_Ctx_1-1.tif");
run("Split Channels");
close();
selectWindow("16779_2_Ctx_1-1.tif (red)");
run("Subtract Background...", "rolling=50");
setAutoThreshold("Default dark");
//run("Threshold...");
run("Select All");
run("Analyze Particles...", "size=4-Infinity pixel display exclude clear
summarize add");
saveAs("Results", "/Users/Justin/Desktop/Results.csv");


Fiji File Edit Language Templates Run Tools Tabs Options
100% Sun Mar 29 11:42 AM Justin Savage
(Fiji Is Just) ImageJ
Dev Stk LUT
(Fiji Is Just) ImageJ 2.0.0-rc-69/1.52p;
Recorder
Record: Macro
Name: Macro.ijm
Create
?
*Macro.ijm.ijm
*Macro.ijm.ijm
1 selectWindow("16779_2_Ctx_1-1.tif");
2 run("Split Channels");
3 close();
4 selectWindow("16779_2_Ctx_1-1.tif (red)");
5 run("Subtract Background...", "rolling=50");
6 setAutoThreshold("Default dark");
7 //run("Threshold...");
8 run("Select All");
9 run("Analyze Particles...", "size=4-Infinity pixel display exclude clear summarize add");
10 saveAs("Results", "/Users/Justin/Desktop/Results.csv");
11
Run
Batch
Kill
persistent
Show Errors
Clear
129.35x129.35 microns (1024x
lude clear
Flatten [F]
More »
Show All
Labels


Fiji File Edit Image Process Analyze Plugins Window Help
(Fiji Is Just) ImageJ
Developer Menu
Macros
Shortcuts
Utilities
New
Compile and Run...
Install... ⇧⌘M
Install PlugIn...
Install...
Run...
Edit...
Startup Macros...
Interactive Interpreter... ⌘J
Record...
129.35x129.35 microns (1024x
//run("Threshold...");
run("Select All");
run("Analyze Particles...", "size=4-Infinity pixel display exclude clear summarize add");
saveAs("Results", "/Users/Justin/Desktop/Results.csv");
Run
Batch
Kill
persistent
Show Errors
Clear
Flatten [F]
More »
Show All
Labels

A macro for one image

```
selectWindow("16779_2_Ctx_1-1.tif");
run("Split Channels");
close();
selectWindow("16779_2_Ctx_1-1.tif (red)");
run("Subtract Background...", "rolling=50");
setAutoThreshold("Default dark");
//run("Threshold...");
run("Select All");
run("Analyze Particles...", "size=4-Infinity pixel display exclude clear summarize add");
saveAs("Results", "/Users/Justin/Desktop/Results.csv");

```

Solving the problem on paper

- Computers can’t do something you don’t know how to do
- Computers are for increasing scale and efficiency

//Comments are your friend

```
1  //selects the first merged image (already Z projected)
2  selectWindow("16779_2_Ctx_1-1.tif");
3  //splits channels
4  run("Split Channels");
5  //closes the blue channel
6  close();
7  //selects the red channel
8  selectWindow("16779_2_Ctx_1-1.tif");
9  //Subtracts the background
10 run("Subtract Background...",  "rolling=50");
11 //Thresholds the image
12 setAutoThreshold("Default dark");
13 //run("Threshold...");
14 run("Select All");
15 //"Analyze Particles" counts the number of puncta
16 run("Analyze Particles...", "size=4-Infinity pixel display exclude clear summarize add");
17 //Saves the puncta
18 saveAs("Results", "/Users/Justin/Desktop/Results.csv");
```

Functions: our tools for computing

```
14 function analyzePuncta(path){
15     //selects the first merged image (already Z projected)
16     selectWindow("16779_2_Ctx_1-1.tif");
17     //splits channels
18     run("Split Channels");
19     //closes the blue channel
20     close();
21     //selects the red channel
22     selectWindow("16779_2_Ctx_1-1.tif");
23     //Subtracts the background
24     run("Subtract Background...",  "rolling=50");
25     //Thresholds the image
26     setAutoThreshold("Default dark");
27     //run("Threshold...");
28     run("Select All");
29     //"Analyze Particles" counts the number of puncta
30     run("Analyze Particles...", "size=4-Infinity pixel display exclude clear summarize add");
31     //Saves the puncta
32     saveAs("Results", "/Users/Justin/Desktop/Results.csv");
33 }
```

```
function analyzePuncta(path){
    //Open the image to be used
    open(path);
    //Gets the name of the image that's open
    currentTitle = getTitle();
    //selects the current merged image (already Z projected)
    selectWindow(currentTitle);
    //splits channels
    run("Split Channels");
    //closes the blue channel
    close(currentTitle + " (blue)");
    //selects the red channel
    selectWindow(currentTitle + " (red)");
    //Subtracts the background
    run("Subtract Background...",  "rolling=50");
    //Thresholds the image
    setAutoThreshold("Default dark");
    run("Threshold...");
    //Gives the user time to threshold the image
    waitForUser("Check threshold");
    //"Analyze Particles" counts the number of puncta
    run("Analyze Particles...", "size=4-Infinity pixel display exclude clear summarize add");
    //Saves the puncta for the red channel
    saveAs("Results", path + "_redResults.csv");
}
```

Analyzing a whole folder

```
1  //Asks user for source directory (aka folder)
2  dirSource  = getDirectory("Choose Source Directory ");
3  //makes a list of the files in that directory
4  listSource = getFileList(dirSource);
5
6  //do the following for each file in the folder
7  for(i = 0; i < listSource.length; i++){
8      //on the "i"th time through the loop,
9      //analyze the "i"th image in the folder
10     currentFile = listSource[i];
11     analyzePuncta(dirSource + currentfile);
12 }
```

listSource is an *array* of *strings*

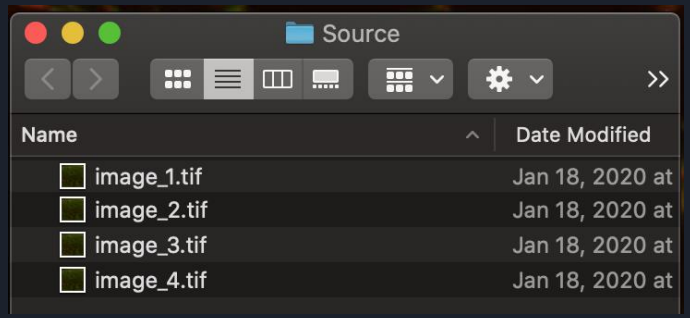
Array - a structured list of things in java

String - a series of characters stored together

“16779_2_Ctx_1-1.tif”

From a folder to an array

```
1 //Asks user for source directory (aka folder)
2 dirSource  = getDirectory("Choose Source Directory ");
3 //makes a list of the files in that directory
4 listSource = getFileList(dirSource);
```



listSource

0	1	2	3
“image_1.tif”	“image_2.tif”	“image_3.tif”	“image_4.tif”

Java counts from 0

listSource

0	1	2	3
“image_1.tif”	“image_2.tif”	“image_3.tif”	“image_4.tif”

listSource[0] = “image_1.tif”

listSource.length = 4

Repeating actions with for loops

```
6  //do the following for each file in the folder
7  for(i = 0; i < listSource.length; i++){
8      //on the "i"th time through the loop,
9      //analyze the "i"th image in the folder
10     currentFile = listSource[i];
11     analyzePuncta(dirSource + currentfile);
12 }
```

Starting at i = 0, do the loop until i = 4 and increase i by 1 each time

“=” is an assignment

In java the “=” sign is used to give something a value

currentFile = listSource[0]

currentFile *gets* “image_1.tif”

String concatenation

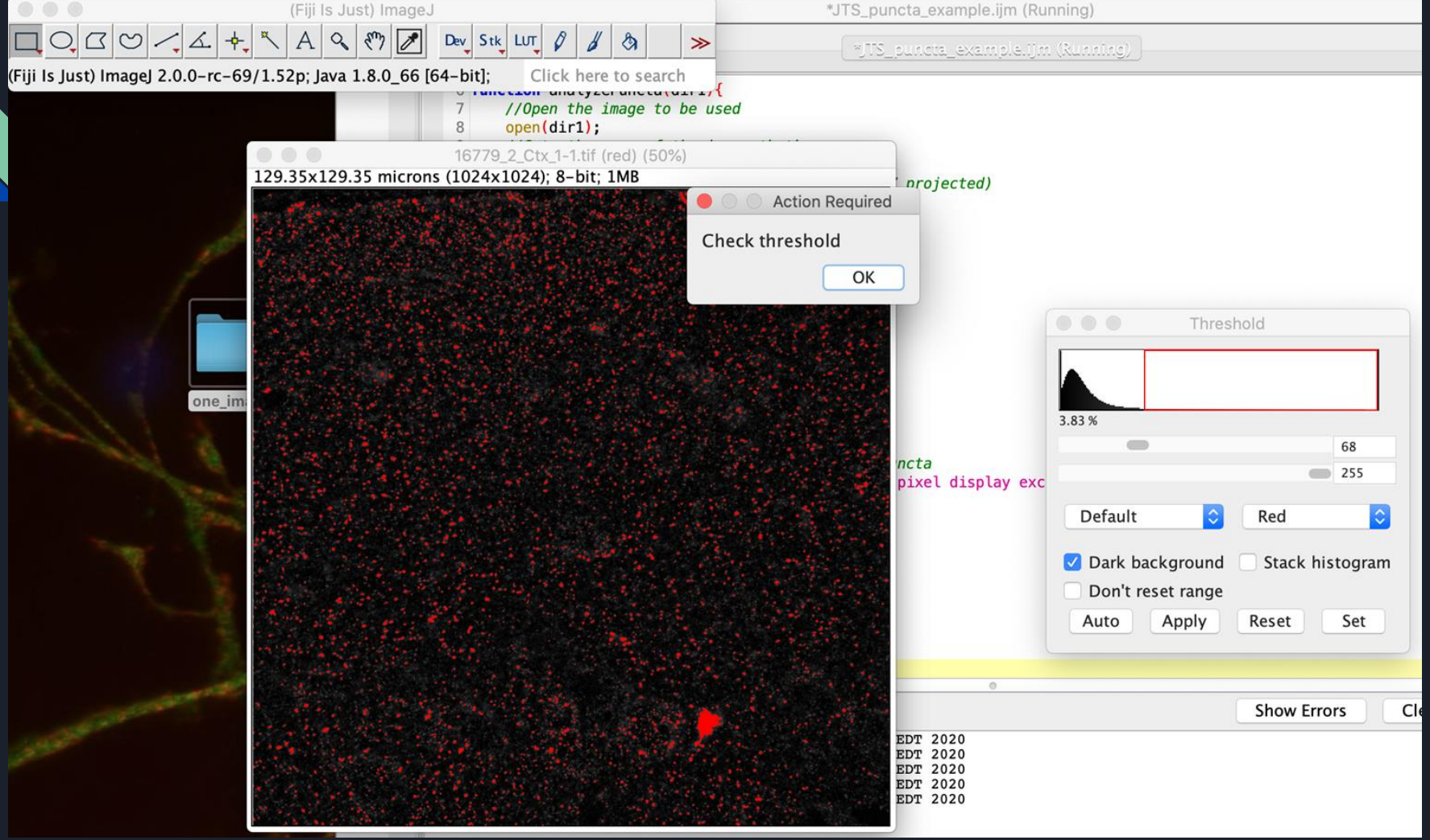
Strings can be combined in a process called concatenation

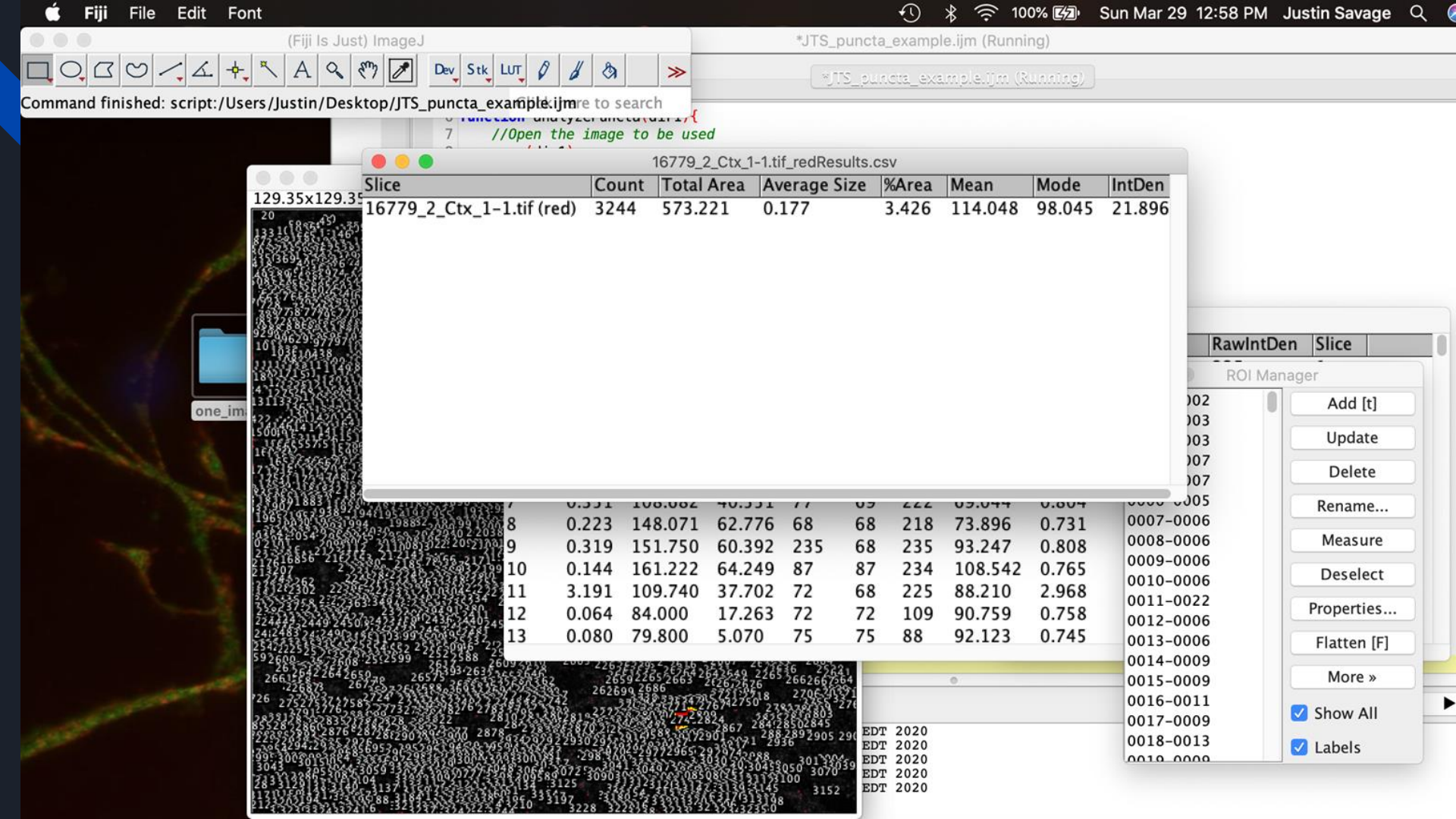
dirSource = “/Users/Justin/Desktop/Source/”

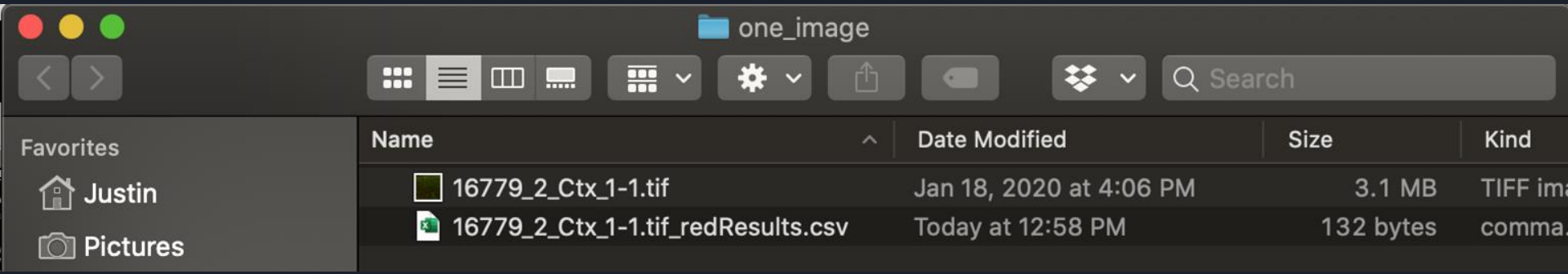
currentFile = “image_1.tif”

dirSource + currentFile = “/Users/Justin/Desktop/Source/image_1.tif”

```
function analyzePuncta(dir1){
    //Open the image to be used
    open(dir1);
    //Gets the name of the image that's open
    currentTitle = getTitle();
    //selects the current merged image (already Z projected)
    selectWindow(currentTitle);
    //splits channels
    run("Split Channels");
    //closes the blue channel
    close(currentTitle + " (blue)");
    //selects the red channel
    selectWindow(currentTitle + " (red)");
    //Subtracts the background
    run("Subtract Background...",  "rolling=50");
    //Thresholds the image
    setAutoThreshold("Default dark");
    run("Threshold...");
    //Gives the user time to threshold the image
    waitForUser("Check threshold");
    //"Analyze Particles" counts the number of puncta
    run("Analyze Particles...", "size=4-Infinity pixel display exclude clear summarize add");
    //Saves the puncta for the red channel
    saveAs("Results", dir1 + "_redResults.csv");
}
//do the following for each file in the folder
for(i = 0; i < listSource.length; i++){
    //on the "i"th time through the loop,
    //analyze the "i"th image in the folder
    currentFile = listSource[i];
    analyzePuncta(dirSource + currentFile);
}
```


(Fiji Is Just) ImageJ
(Fiji Is Just) ImageJ 2.0.0-rc-69/1.52p; Java 1.8.0_66 [64-bit];
Click here to search
*JTS_puncta_example.ijm (Running)
7 //Open the image to be used
8 open(dir1);
16779_2_Ctx_1-1.tif (red) (50%)
129.35x129.35 microns (1024x1024); 8-bit; 1MB
Action Required
Check threshold
OK
Threshold
3.83 %
68
255
Default
Red
Dark background
Stack histogram
Don't reset range
Auto
Apply
Reset
Set
Show Errors
EDT 2020
EDT 2020
EDT 2020
EDT 2020
EDT 2020


Fiji File Edit Font
100% Sun Mar 29 12:58 PM Justin Savage
(Fiji Is Just) ImageJ
*JTS_puncta_example.ijm (Running)
Command finished: script:/Users/Justin/Desktop/JTS_puncta_example.ijm
16779_2_Ctx_1-1.tif_redResults.csv
Slice Count Total Area Average Size %Area Mean Mode IntDen
16779_2_Ctx_1-1.tif (red) 3244 573.221 0.177 3.426 114.048 98.045 21.896
RawIntDen Slice
ROI Manager
Add [t]
Update
Delete
Rename...
Measure
Deselect
Properties...
Flatten [F]
More »
Show All
Labels
0007-0006
0008-0006
0009-0006
0010-0006
0011-0022
0012-0006
0013-0006
0014-0009
0015-0009
0016-0011
0017-0009
0018-0013

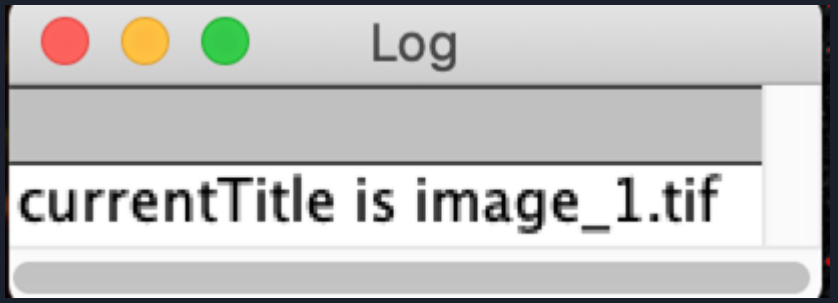

one_image
Search
Favorites
Justin
Pictures
Name
Date Modified
Size
Kind
16779_2_Ctx_1-1.tif
Jan 18, 2020 at 4:06 PM
3.1 MB
TIFF ima
16779_2_Ctx_1-1.tif_redResults.csv
Today at 12:58 PM
132 bytes
comma

What’s next?

- Do the same for the green channel
- Close windows when we’re done with them
- Use puncta data to calculate colocalizations

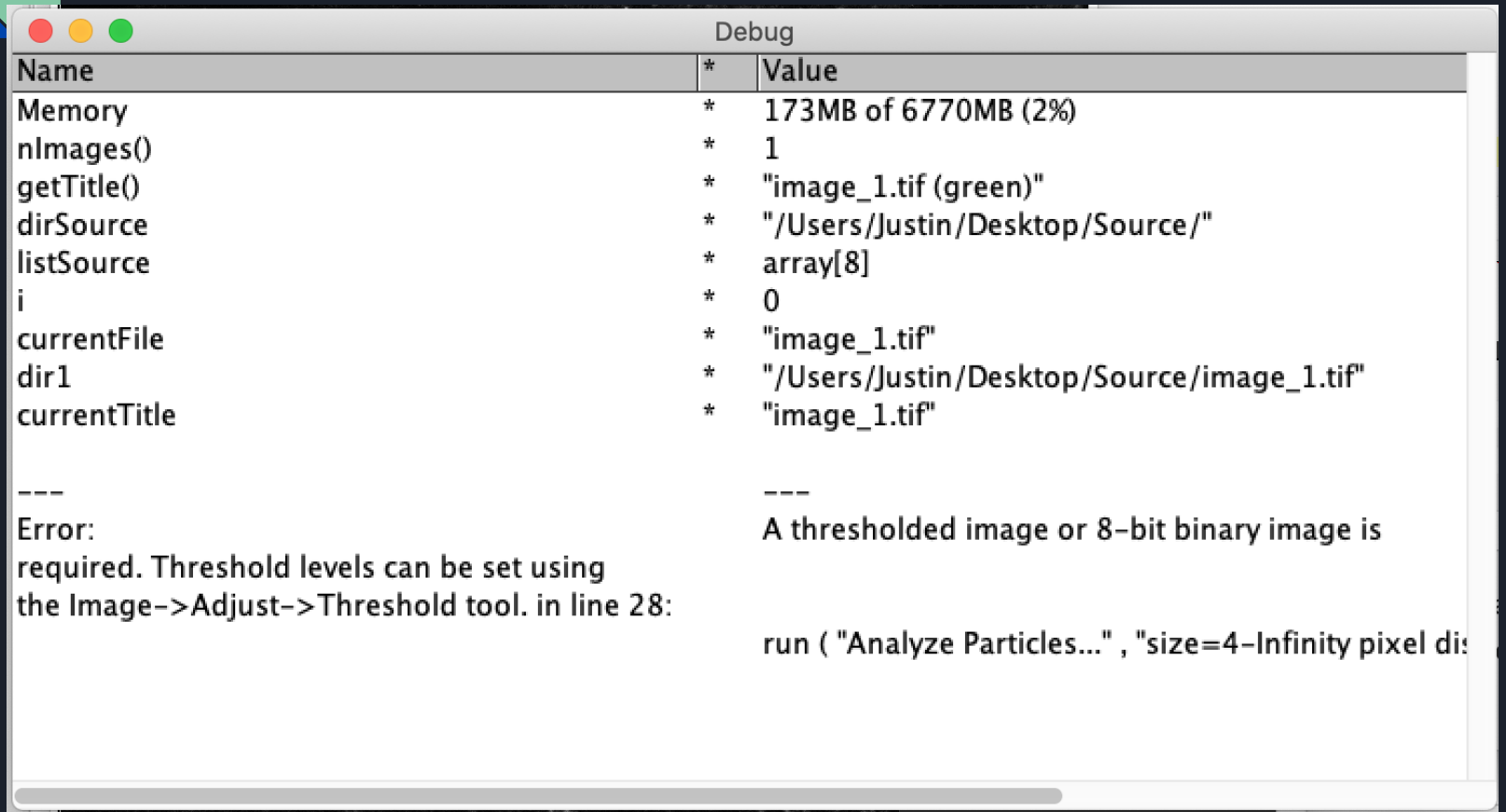
What if something goes wrong?: Print

```
//Gets the name of the image that's open
currentTitle = getTitle();
print("currentTitle is " + currentTitle);
```



What if something goes wrong?: Error Message

What if something goes wrong?: Debug



Where to go for help

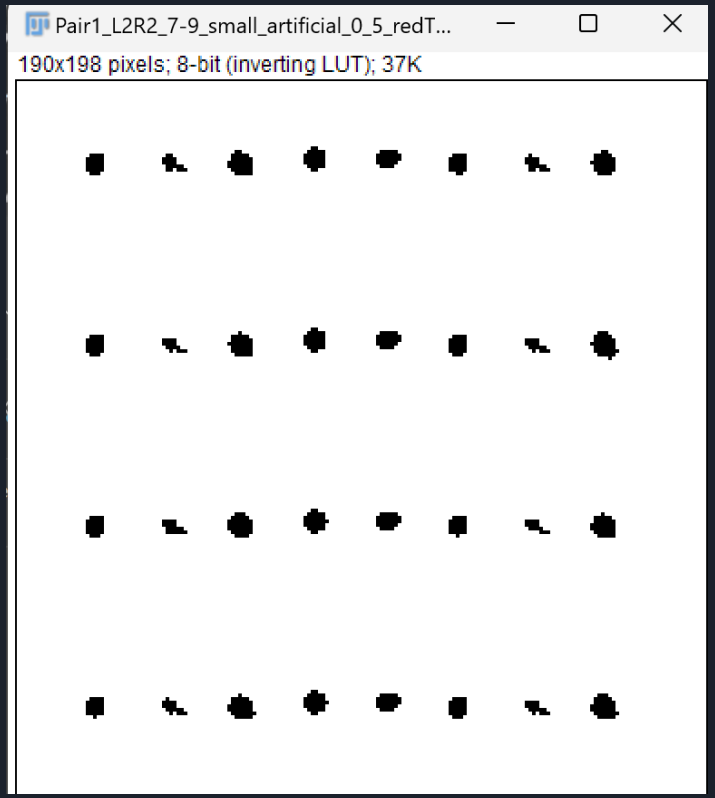
- ImageJ Website: https://imagej.net/Welcome
  - Has lots of info on every imagej function and how to download new ones
- ImageJ macro functions list: https://imagej.nih.gov/ij/developer/macro/functions.html
  - Can help to find functions that didn't come up in the recorder
- Image.sc Forum https://forum.image.sc/
- *The Digital Cell* (Cold Spring Harbor Labs) https://www.cshlpress.com/default.tpl?cart=15855937407242452 12&fromlink=T&linkaction=full&linksortby=oop_title&--eqSKUdatarq=1282
- Google: Someone else has probably ran into a similar issue
- Email me: justin.savage@duke.edu

Advanced application: ilastik thresholding

- The ilastik team has an ImageJ plugin
- It can be used as is or modified for easier saving of output images

```
16  run("Configure ilastik for Syn_Bot", "executablefile=["+ilastikDir+"] numthreads=-1 maxrammb=4096"
    );
17
18  run("Run Pixel Classification Prediction for Syn_Bot", "projectfilename=["+ilpDir+
    "] saveonly=false inputimage=["+title+"] pixelclassificationtype=Probabilities");
```

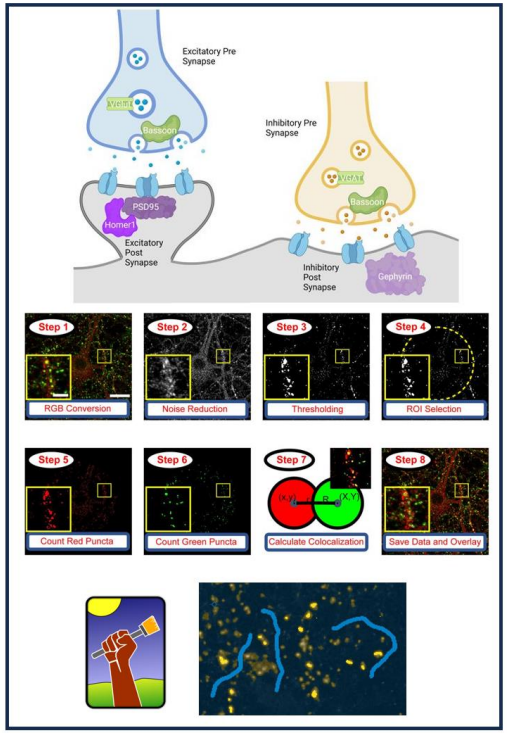
Advanced application: ilastik thresholding

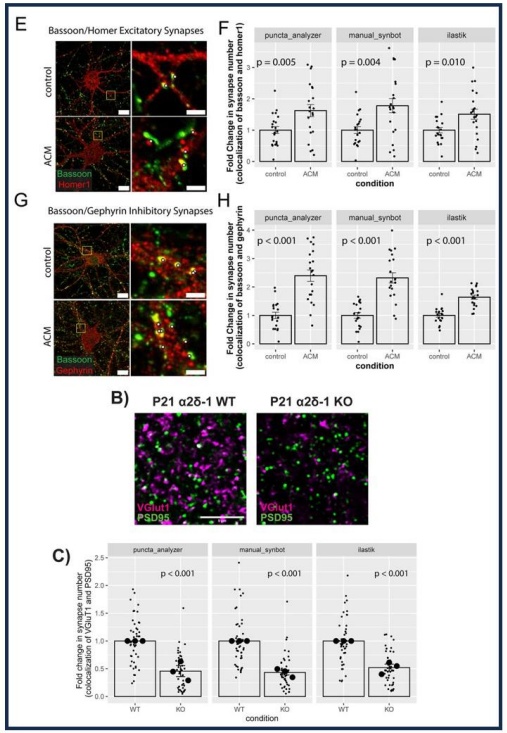


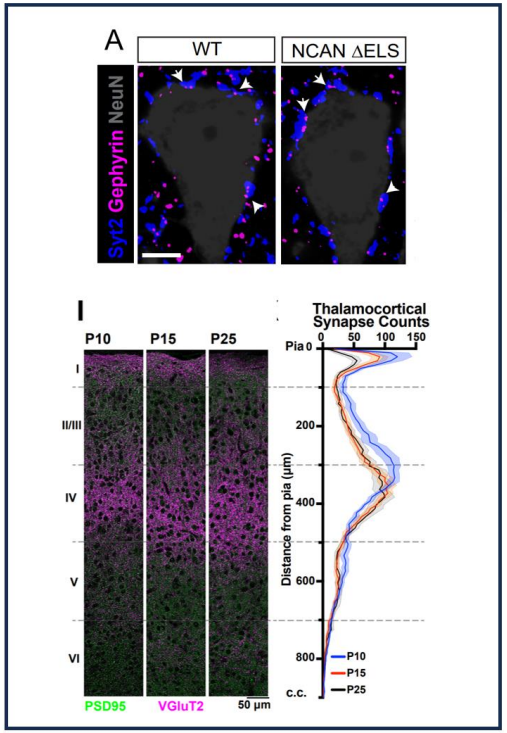
Advanced application: SynBot

SynBot: An open-source image analysis software for automated quantification of synapses

Justin T. Savage, Juan Ramirez, W. Christopher Risher, Dolores Irala, Cagla Eroglu







Getting input from the User

- `waitForUser(string);`
- Dialog box

Getting input from the User

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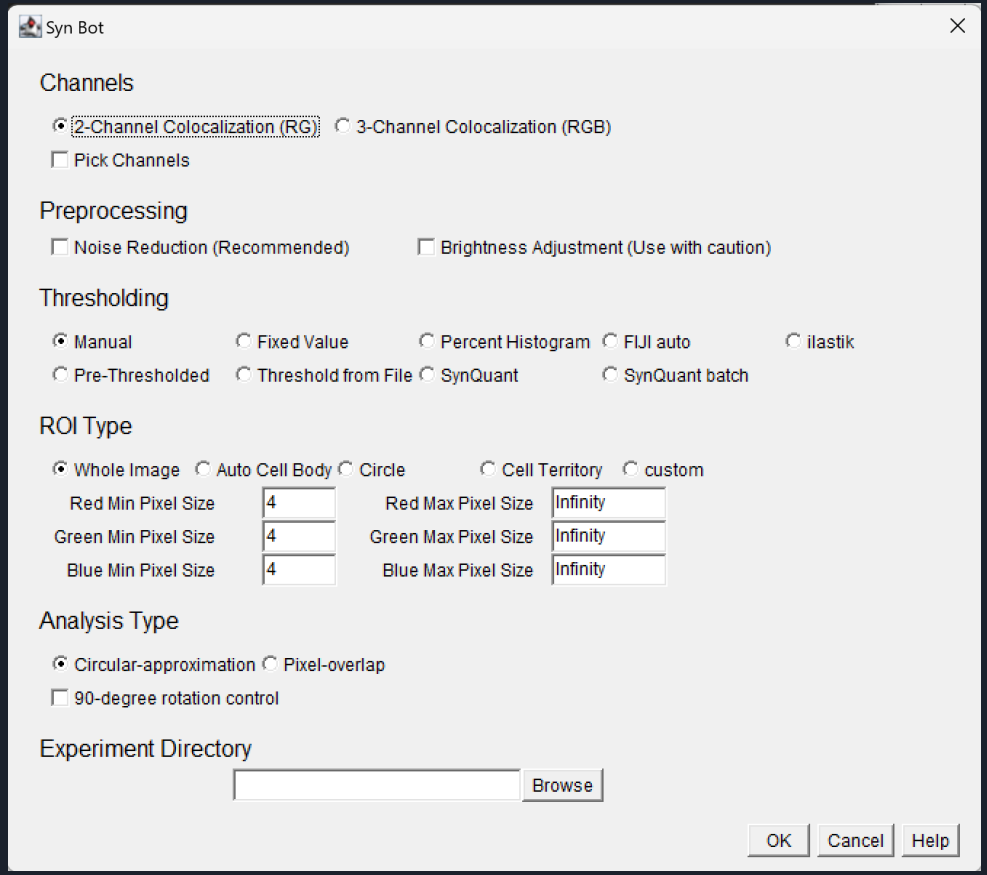

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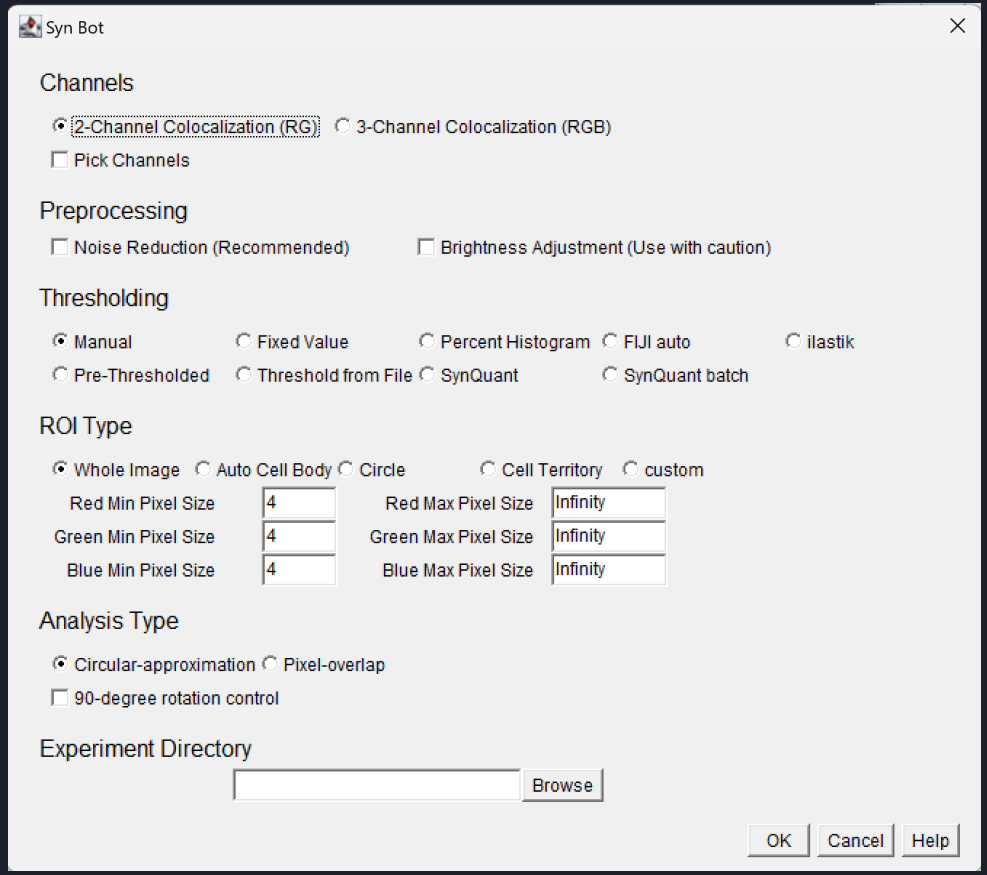

Getting input from the User

```
if (threshType == "Fixed Value"){
    Dialog.create("Fixed Threshold Value");
    Dialog.addNumber("Lower Red Threshold Value", 70);
    Dialog.addNumber("Lower Green Threshold Value", 70);
    Dialog.addNumber("Lower Blue Threshold Value", 70);
    Dialog.show();
    setRedT = Dialog.getNumber();
    setGreenT = Dialog.getNumber();
    setBlueT = Dialog.getNumber();
}
```

Getting input from the User

```
if (threshType == "Fixed Value"){
    Dialog.create("Fixed Threshold Value")
    Dialog.addNumber("Lower Red Threshold
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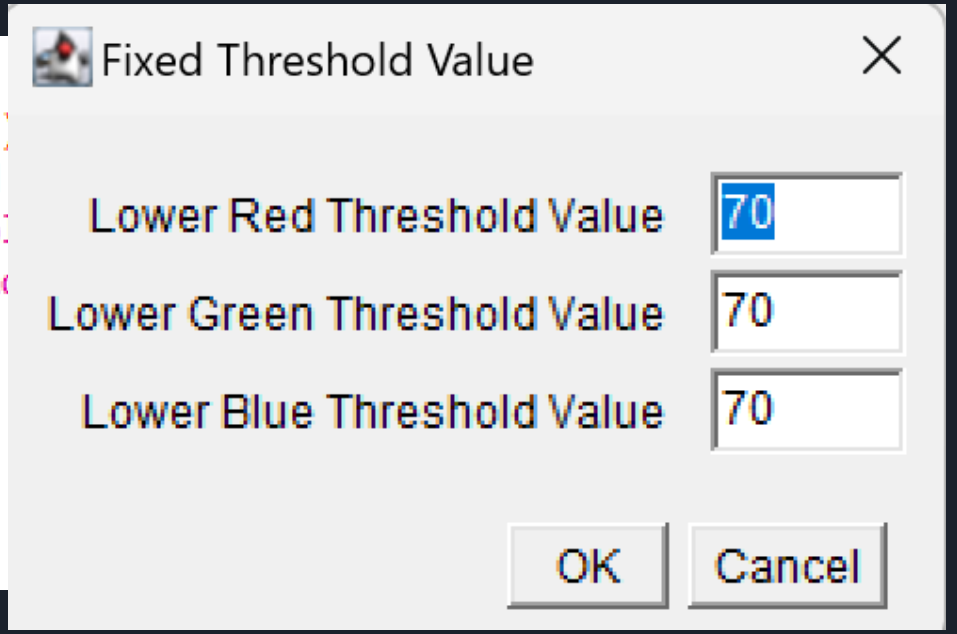
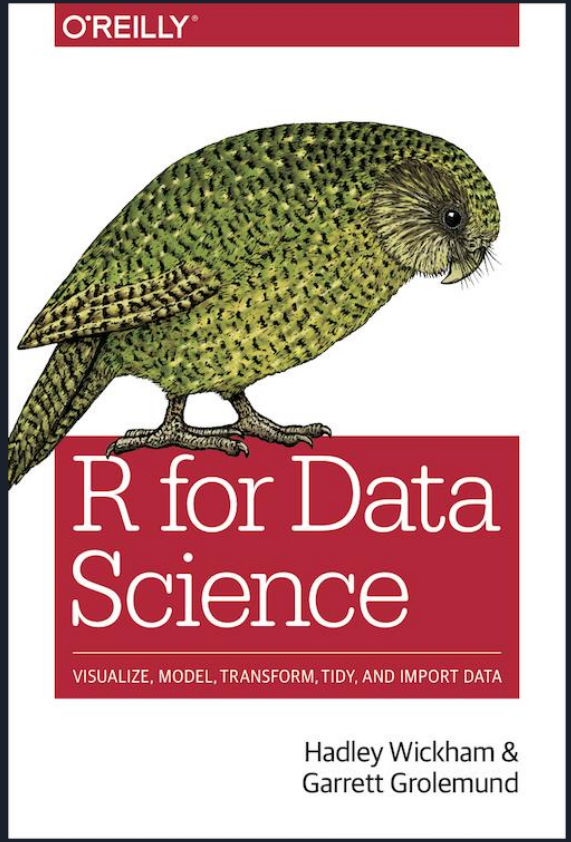


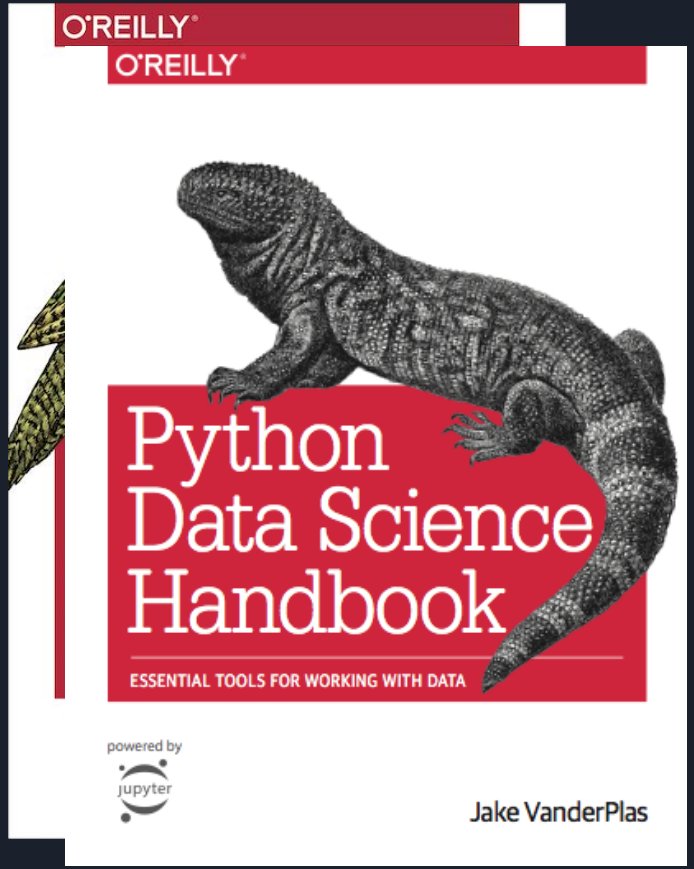
What to do with spreadsheet data after ImageJ

- Use R!!!!!
- https://r4ds.had.co.nz/



What to do with spreadsheet data after ImageJ

- Use R!!!!!
- https://r4ds.had.co.nz/
- Maybe use Python with pandas
- https://github.com/jakevdp/PythonDataScienceHandbook



Additional Resources

Biocoding Resources
https://bit.ly/Biocoding

SynBot
BioRxiv