

Outline

- What is ImageJ? (& what is Fiji?)
- Image Analysis with ImageJ
- Scripting with ImageJ
- ImageJ with OMERO

What is ImageJ?

- A tool for *scientific* image analysis
- Free, Open Source software
- Rich ecosystem: thousands of [plugins](#)
- Easy to extend and easy to contribute; large community

The main ImageJ window

Menubar

Tools

Status Bar



Fiji Is Just ImageJ

- Based on ImageJ
- Comes with plugins useful for the **Life Sciences**
- Has an updater and many optional update sites
- Has additional developer features (easier to write scripts, develop new plugins)

Help: finding commands & updates

The screenshot displays the Fiji software interface. The main window is titled "(Fiji Is Just) ImageJ" and shows a toolbar with various tools. A "Command Finder" dialog box is open, listing commands and their menu paths. The "Help" menu is also open, showing options like "Search", "ImageJ Website...", "Update ImageJ...", and "About Plugins".

Command Finder

| Command | Menu Path | Class | File |
|-----------------------------------|------------------------------|-----------------------------------|------------|
| Load Particles (.tif file - fast) | File>Import>QuickPALM | QuickPALM.Load_particles_table... | /Applic... |
| Open VirtualStack | File>Import>QuickPALM | QuickPALM.Run_MyMacro("Fast_... | /Applic... |
| Save Particles (.tif file - fast) | File>Import>QuickPALM | QuickPALM.Save_particles_table... | /Applic... |
| *recent commands | Plugins>Shortcuts | fiji.util.Recent_Commands | |
| /Users/pwalczysko/Desktop/Ba... | File>Open Recent | | |
| /Users/pwalczysko/Desktop/Sc... | File>Open Recent | | |
| /Users/pwalczysko/Downloads/... | File>Open Recent | | |
| /Users/pwalczysko/Downloads/... | File>Open Recent | | |
| /Users/pwalczysko/Downloads/... | File>Open Recent | | |
| /Users/pwalczysko/Downloads/... | File>Open Recent | | |
| 16 colors | Image>Lookup Tables | | |
| 16-bit | Image>Type | ij.plugin.Converter("16-bit") | |
| 2D Histogram | Plugins>Analyze | util.Histogram_2D | /Applic... |
| 2D Stitching | Plugins>Stitching>deprecated | Stitching_2D | /Applic... |
| 3-3-2 RGB | Image>Lookup Tables | ij.plugin.LutLoader("3-3-2 RGB") | |
| 32-bit | Image>Type | ij.plugin.Converter("32-bit") | |
| 3D OC Options | Analyze | _3D_OC_Options | /Applic... |
| 3D Objects Counter | Analyze | _3D_objects_counter | /Applic... |

Close window after running command

Run Source Close Help

Help

Search

ImageJ Website...
ImageJ News...
Documentation...
Installation...
Mailing List...

Dev. Resources...
Plugins...
Macros...
Macro Functions...
Examples

Update ImageJ...
Refresh Menus

About Plugins
About ImageJ...

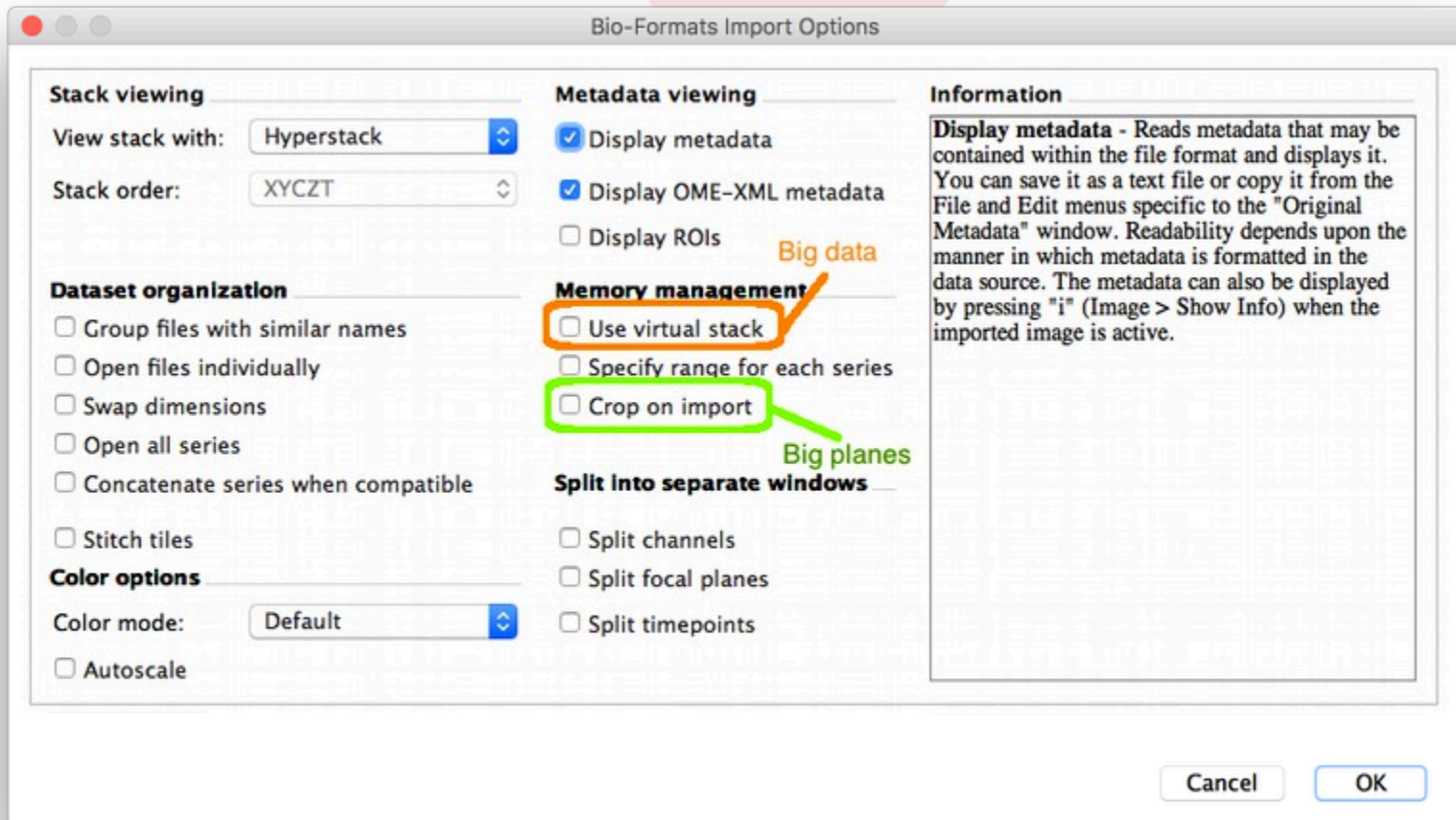
Report a Bug
Help on Menu Item
Licenses
Switch to Modern Mode
Fiji Wiki
Update Fiji

Update...
Upload Sample Image

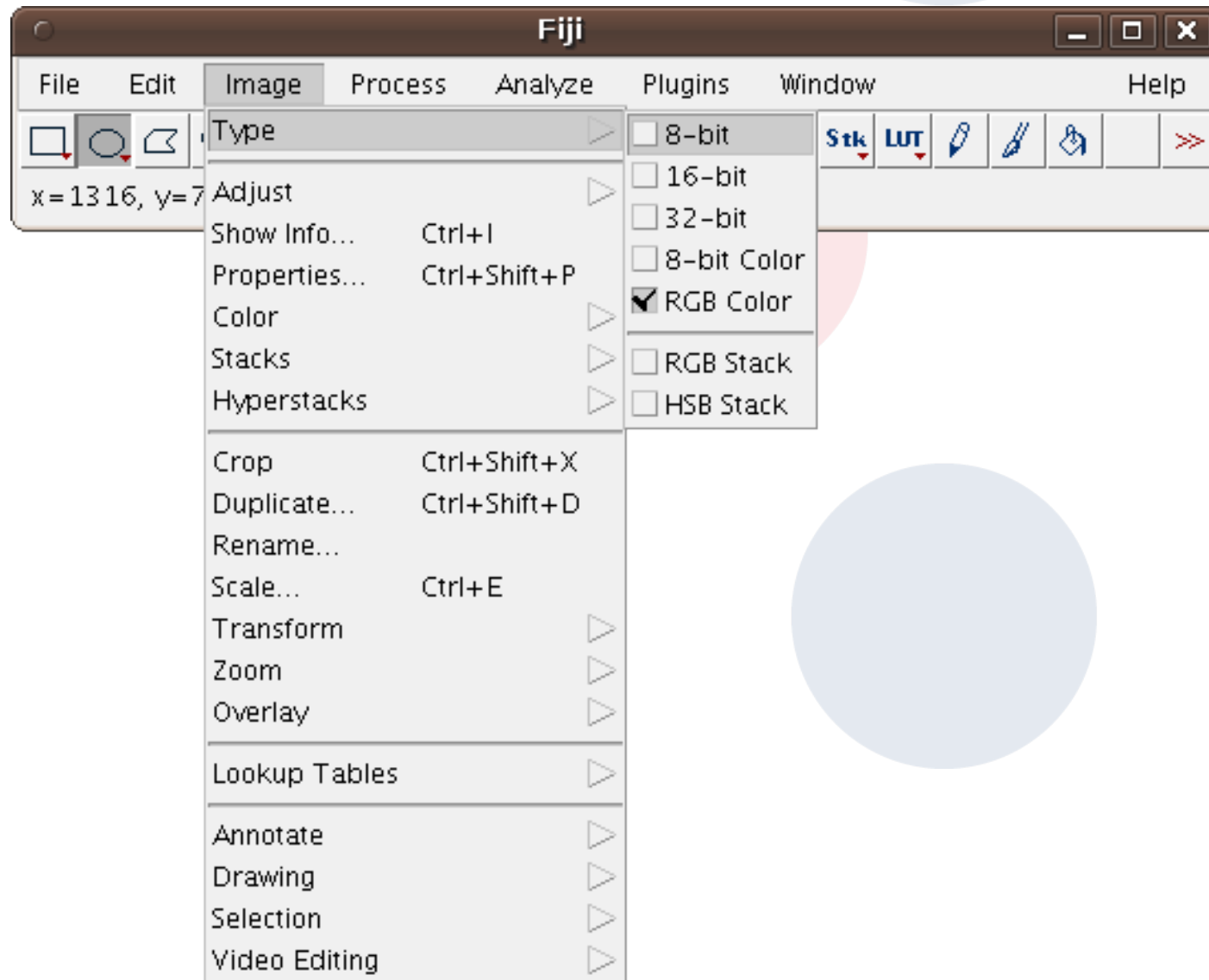
- *Ctrl + I* (“look up” command)
- or *Help* ▶ *Search* ▶ Type into the box

Opening data (and Bio-Formats)

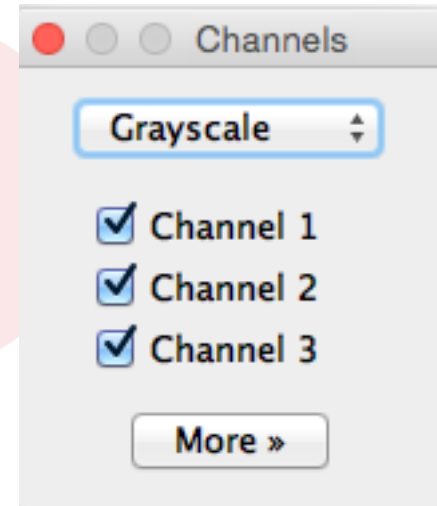
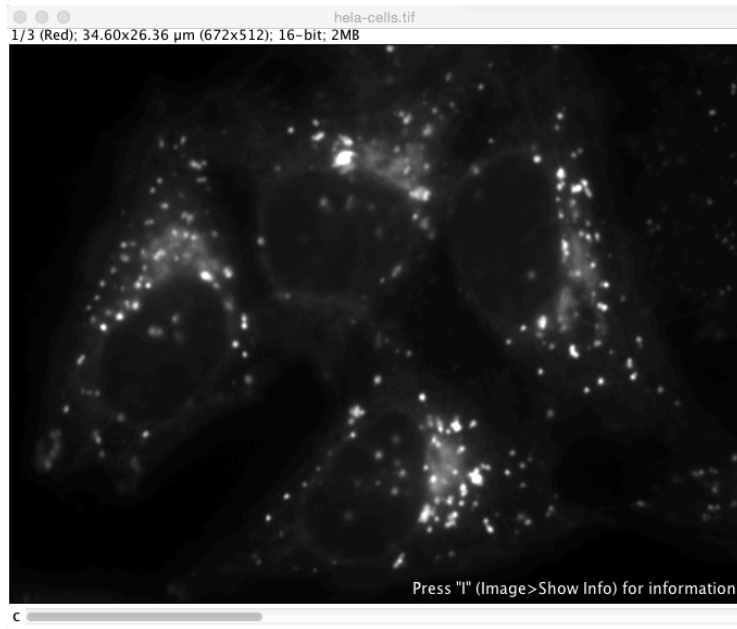
- Drag and Drop
- File > Open
- File > Import > Bio-Formats
- Plugins > Bio-Formats > Bio-Formats Importer



Pixel Types



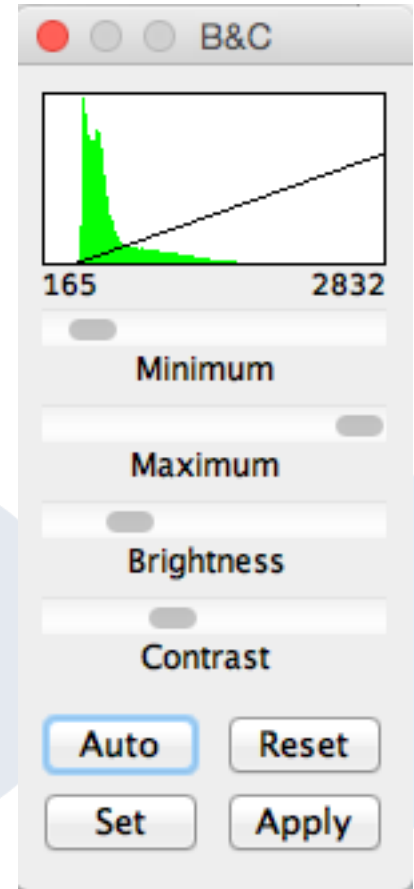
Channels Tool



- Switch Channels on and off, adjust Brightness and Contrast
- *File* ▶ *Open Samples* ▶ *HeLa*
- *Image* ▶ *Color* ▶ *Channels Tool* (Shift + Z) ▶ *Grayscale*
- Switch to Color, then switch to Composite
- *More* ▶ *Properties* ▶ *Convert to RGB* for publication or output

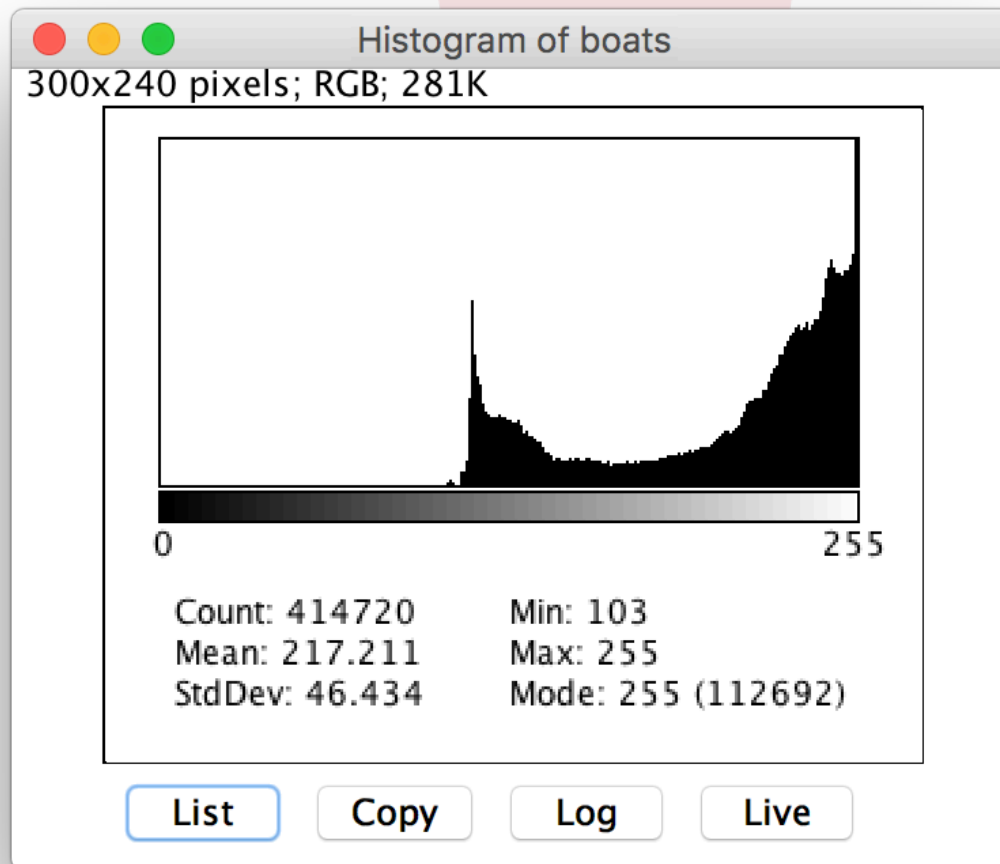
Adjust Brightness and Contrast

- *Image > Adjust > Brightness and Contrast*
- *(how measured intensities are mapped to display intensities)*



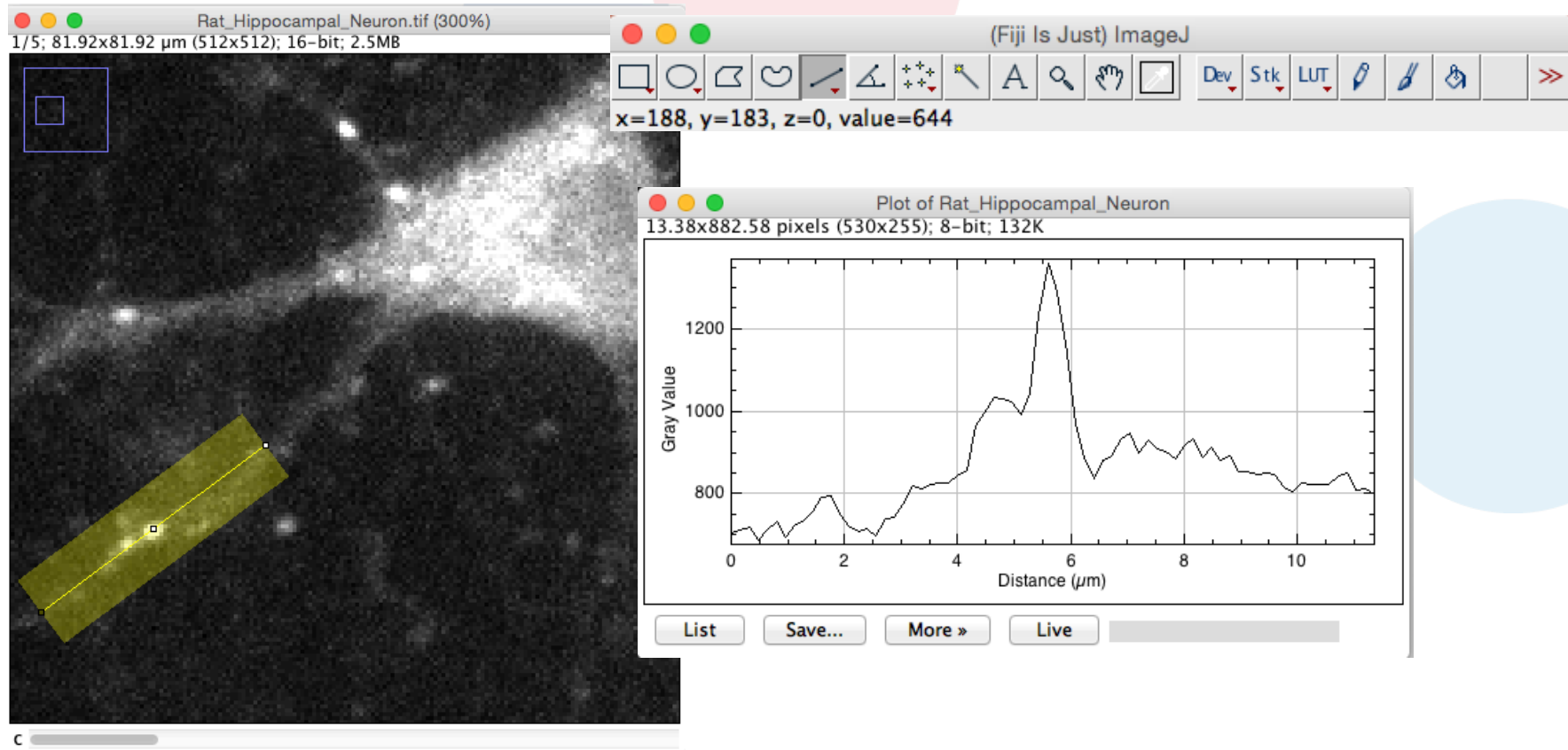
Histogram

- *Analyze > Histogram*
- i.e. frequency/count for each intensity level



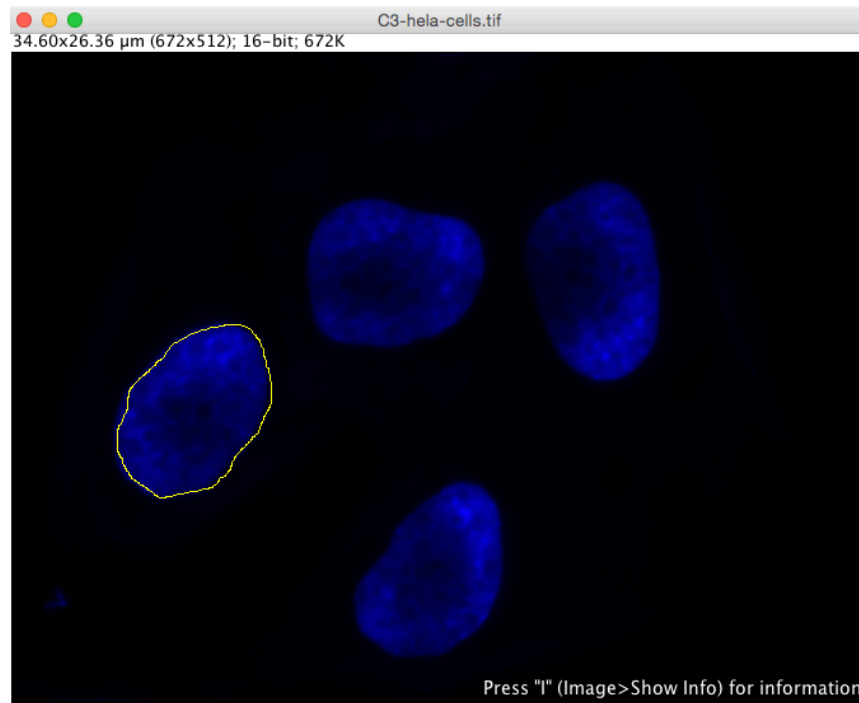
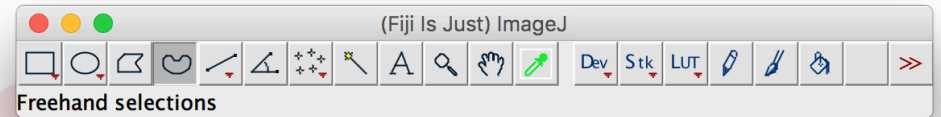
Profile Plots

- Use any Line tool
- Analyze > Plot Profile
- E.g. estimate signal-to-noise for feature tracking

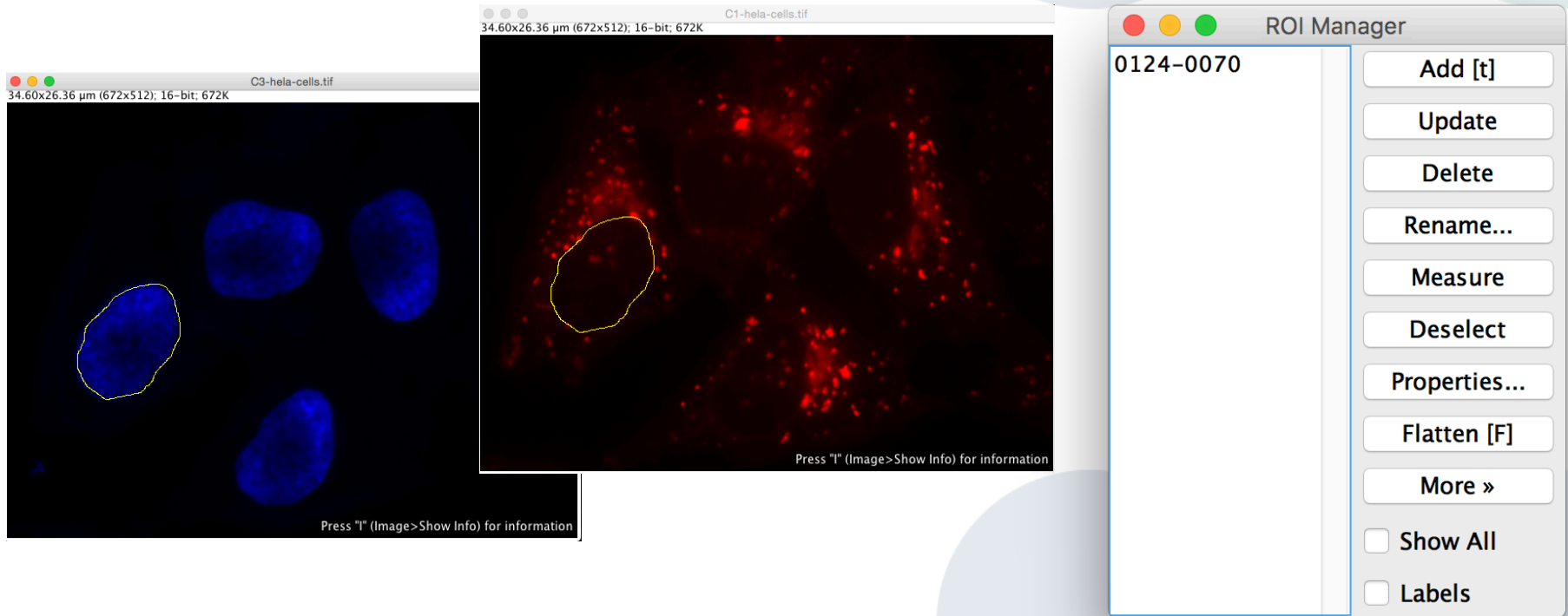


Regions of Interest (ROI)

- *File* ▶ *Open Samples* ▶ *HeLa*
- *Image* ▶ *Color* ▶ *Split Channels*
- Selections: e.g. Freehand selection tool
- How can a blue channel nucleus selection be copied to the red channel?



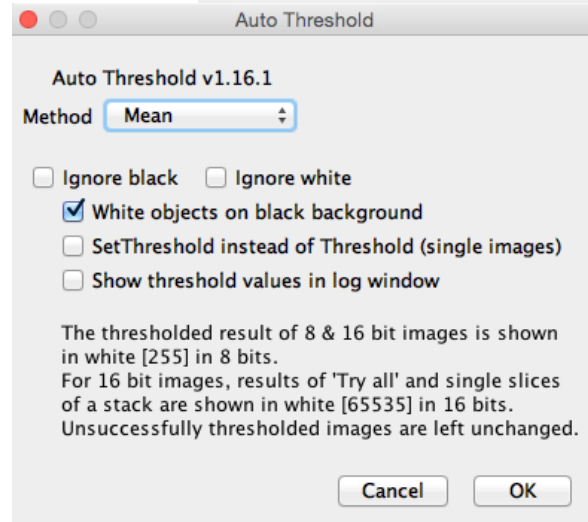
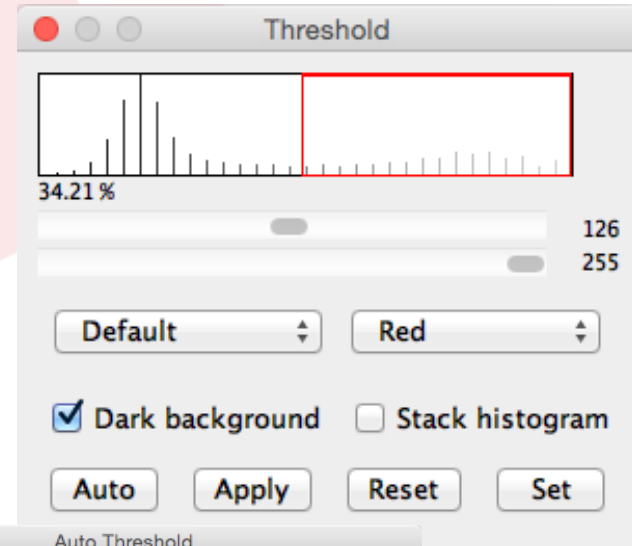
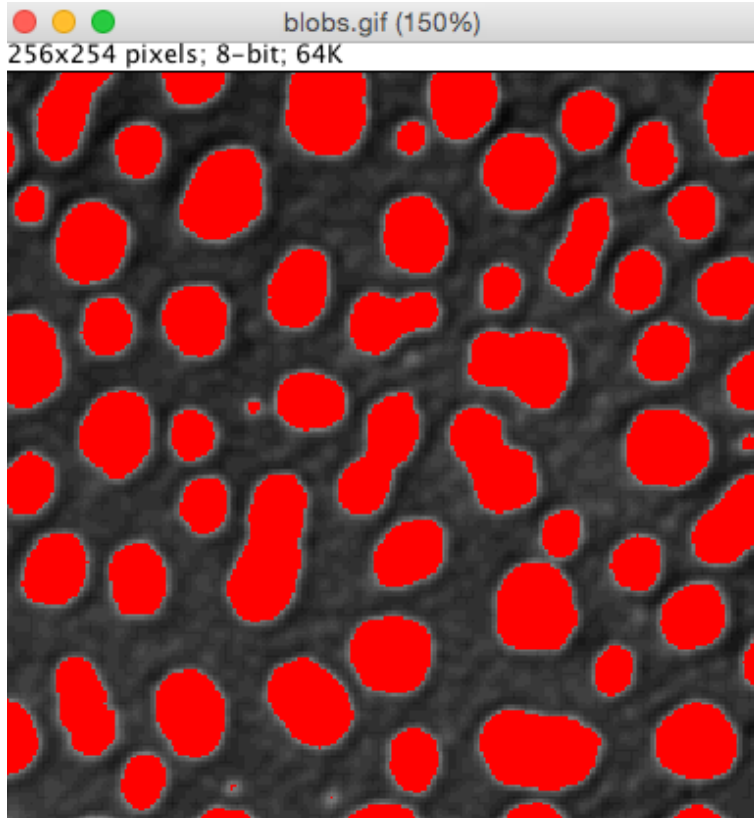
The ROI Manager: a “clipboard” for ROIs



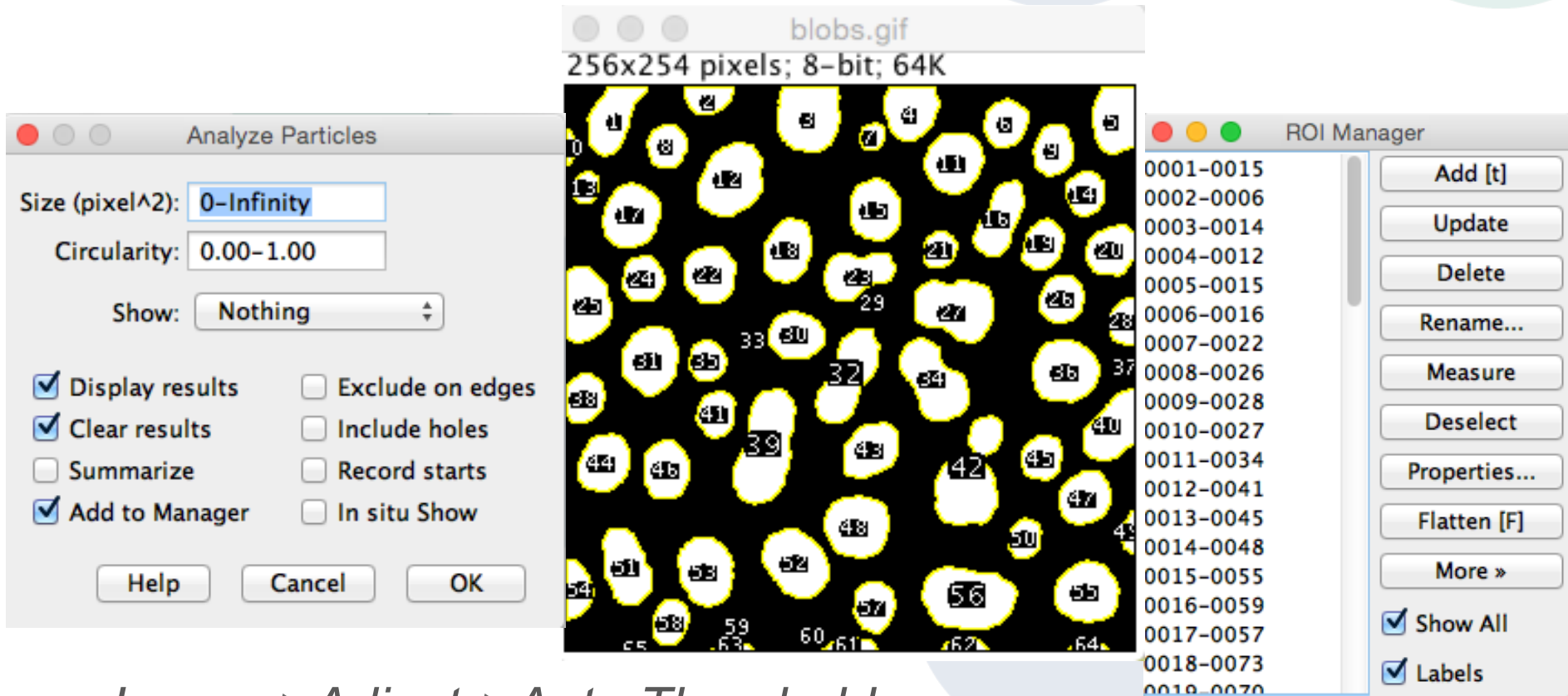
- Select the image with a ROI
- Press “T” key – **or** *Analyze* ▶ *Tools* ▶ *ROI Manager*
- Select other image
- Click ROI in manager – **or** *Edit* ▶ *Selection* ▶ *Restore Selection*

Segmentation (by intensity threshold)

- *Image* ▶ *Adjust* ▶ *Threshold...* (Shift + T)
- *Image* ▶ *Adjust* ▶ *Auto Threshold...* (Shift + T)



Identifying, labeling & measuring objects

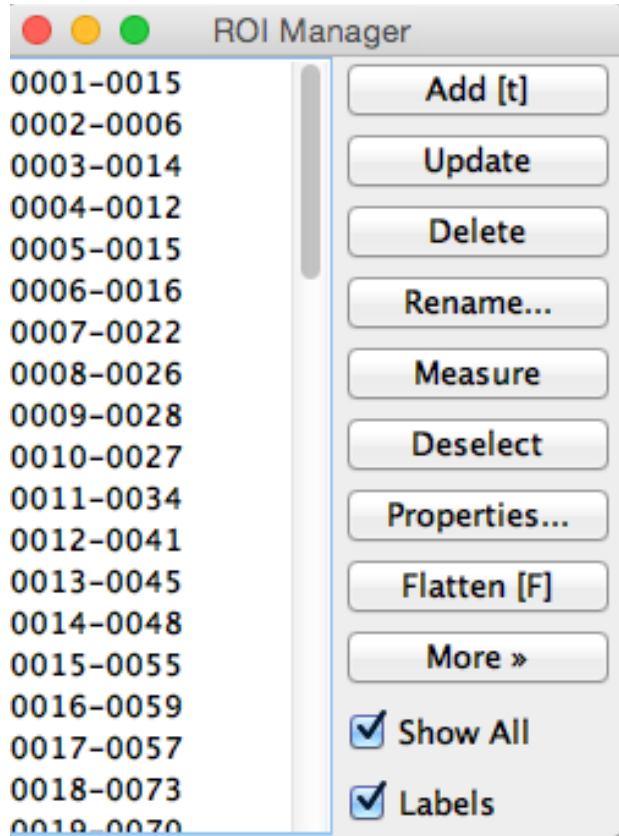


○ *Image* ▶ *Adjust* ▶ *Auto Threshold*

○ *Analyze* ▶ *Analyze Particles...*

- *Display results* checkbox is checked -> Results table appears

Results table



The Results table window displays a table with 10 rows and 5 columns: Area, Mean, Min, and Max. The data is as follows:

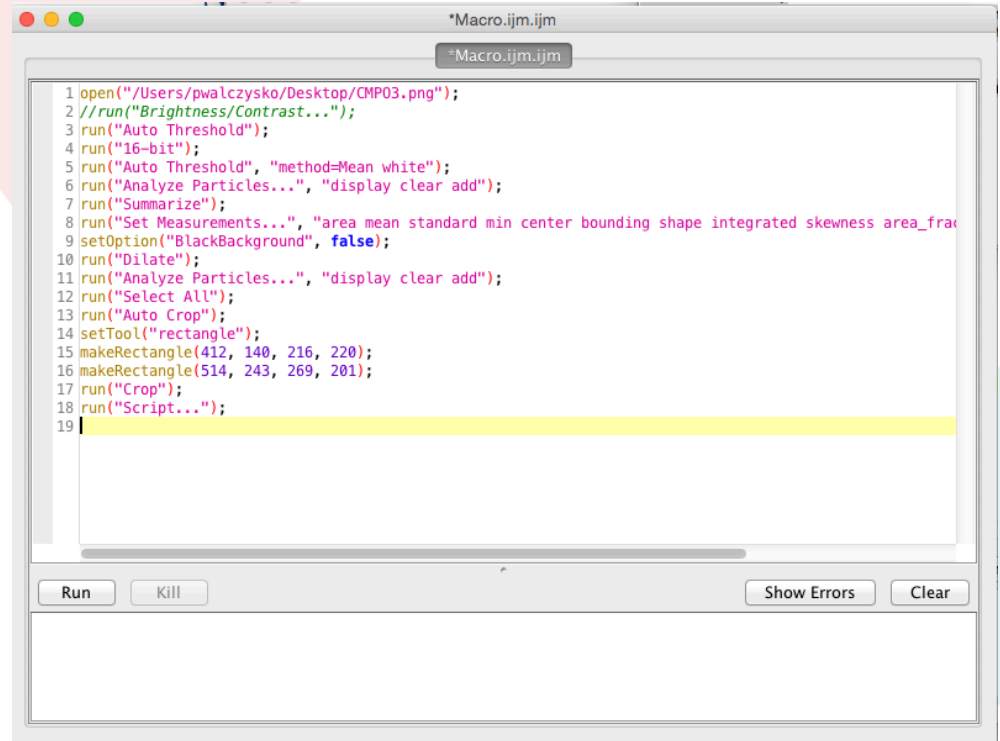
| | Area | Mean | Min | Max |
|----|------|------|-----|-----|
| 1 | 488 | 255 | 255 | 255 |
| 2 | 213 | 255 | 255 | 255 |
| 3 | 709 | 255 | 255 | 255 |
| 4 | 477 | 255 | 255 | 255 |
| 5 | 501 | 255 | 255 | 255 |
| 6 | 321 | 255 | 255 | 255 |
| 7 | 105 | 255 | 255 | 255 |
| 8 | 268 | 255 | 255 | 255 |
| 9 | 316 | 255 | 255 | 255 |
| 10 | 45 | 255 | 255 | 255 |

- Populated by analysis functions, e.g. *Analyze particles* or
- *Analyze* ► *Measure* (shortcut key: 'm' for measure)
- Configure *what* is measured : *Analyze* ► *Set Measurements*

Benefits of Scripting

Facilitates reproducible science:

- Automate analysis
- Document your work
- Share workflows



The image shows a screenshot of the Fiji Script Editor window. The window title is "*Macro.ijm.ijm". The script content is as follows:

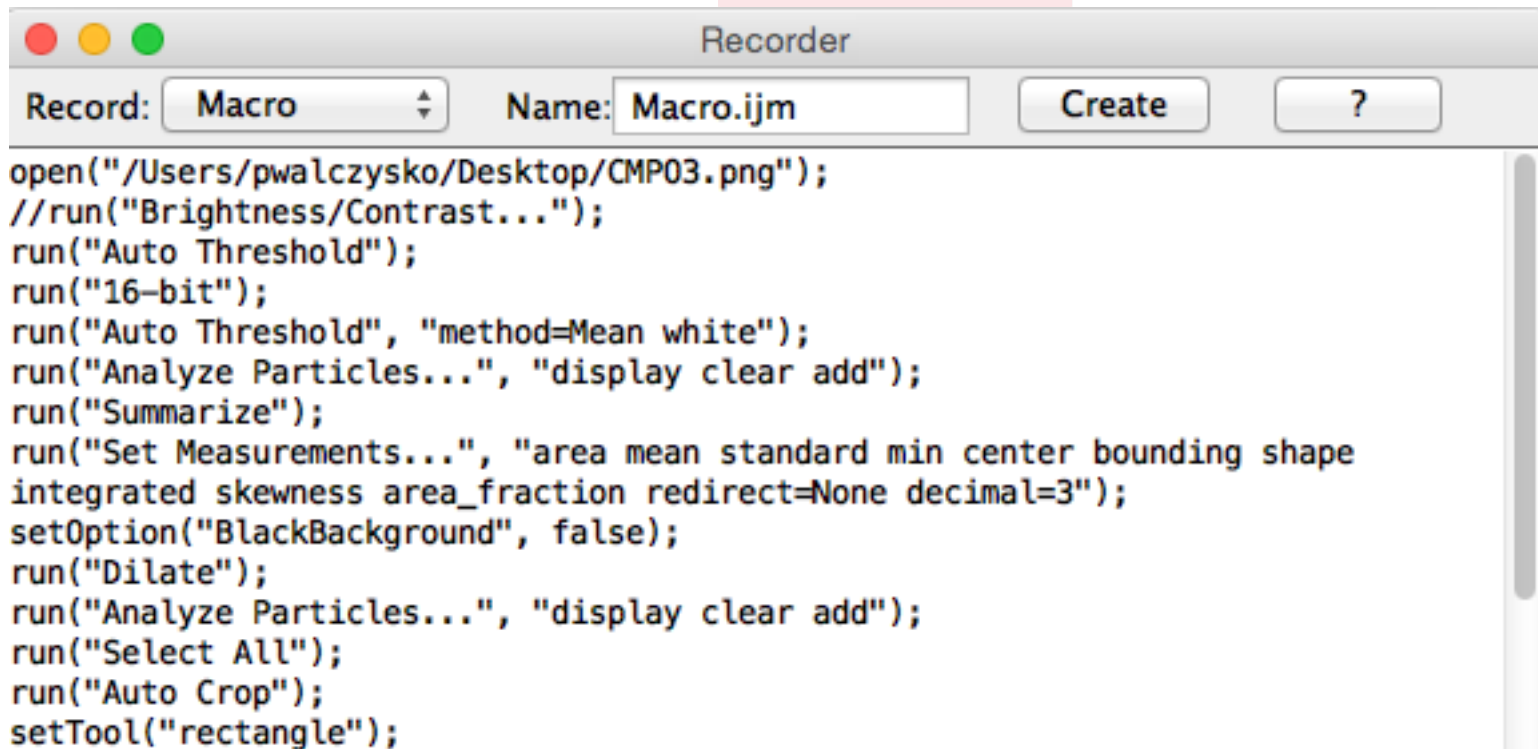
```
1 open("/Users/pwalczysko/Desktop/CMPO3.png");
2 //run("Brightness/Contrast...");
3 run("Auto Threshold");
4 run("16-bit");
5 run("Auto Threshold", "method=Mean white");
6 run("Analyze Particles...", "display clear add");
7 run("Summarize");
8 run("Set Measurements...", "area mean standard min center bounding shape integrated skewness area_fra
9 setOption("BlackBackground", false);
10 run("Dilate");
11 run("Analyze Particles...", "display clear add");
12 run("Select All");
13 run("Auto Crop");
14 setTool("rectangle");
15 makeRectangle(412, 140, 216, 220);
16 makeRectangle(514, 243, 269, 201);
17 run("Crop");
18 run("Script...");
19 |
```

The script is displayed in a text area with a yellow highlight on the last line. Below the text area are buttons for "Run", "Kill", "Show Errors", and "Clear".

the Fiji Script Editor

Macros are easy in ImageJ

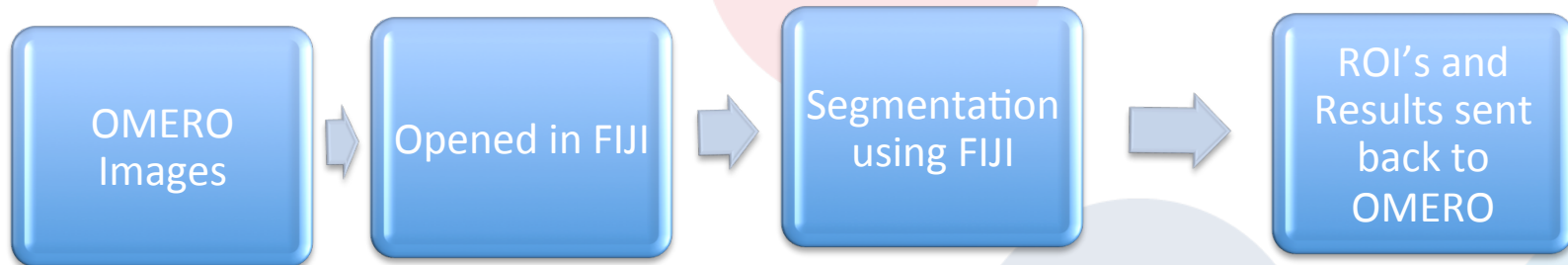
- *Plugins* ▶ *Macros* ▶ *Record...*
- Execute a commands sequence, then click *Create*



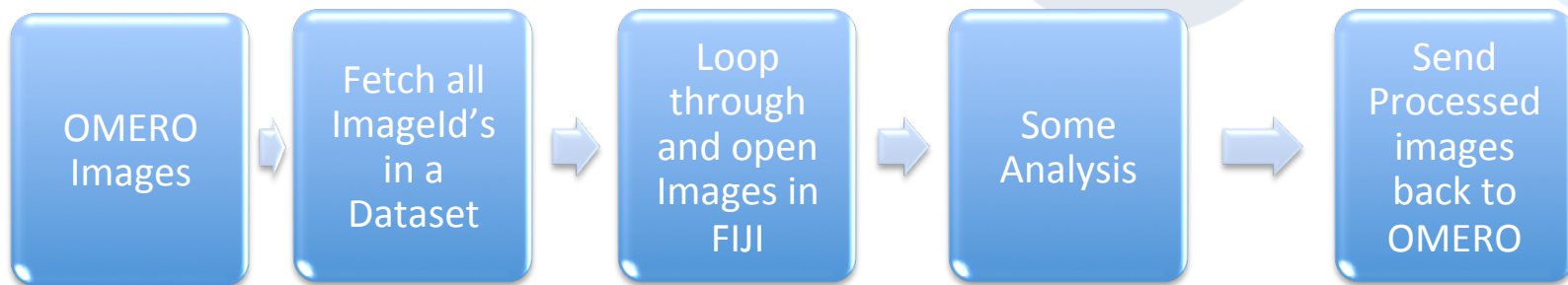
```
open("/Users/pwalczysko/Desktop/CMP03.png");
//run("Brightness/Contrast...");
run("Auto Threshold");
run("16-bit");
run("Auto Threshold", "method=Mean white");
run("Analyze Particles...", "display clear add");
run("Summarize");
run("Set Measurements...", "area mean standard min center bounding shape
integrated skewness area_fraction redirect=None decimal=3");
setOption("BlackBackground", false);
run("Dilate");
run("Analyze Particles...", "display clear add");
run("Select All");
run("Auto Crop");
setTool("rectangle");
```

FIJI-OMERO Workflow Outline

- Setup OMEROIJ Plugin
- Manual Workflow:



- Batch Analysis using FIJI scripts:



OMEROIJ Plugin : Setup

Dependency : OMERO IJ Plugin



OMERO 5.2.0 Downloads

[Clients](#) | [Plugins](#) | [Additional](#) | [Servers](#) | [API](#) | [Python](#) | [Java](#) | [Code](#) | [Components](#) | [Previous versions](#)

- Information on this release of OMERO is in the [release announcement](#)
- Full documentation is available as [web documentation](#) or [PDF documentation](#) and there are user guides for the clients on our [Help website](#)
- A standard OMERO user just needs to download the client package with the same major version as their institutional server e.g. 5.0 clients with the 5.0 server
- If you do not have an institutional server, you can apply for an [account on our Demo server](#) or download the [Virtual Appliance](#) to install your own version locally.

OMERO client downloads

| Client | Size | File Name | Checksum |
|----------|----------|---|-----------------|
| Windows | 83.02 MB | OMERO.clients-5.1.0-ice35-b101.win.zip | 5712f4bc (SHA1) |
| Mac OS X | 82.8 MB | OMERO.clients-5.2.0-ice35-b101.mac_Java7+.zip | 8dae773c (SHA1) |
| Linux | 82.67 MB | OMERO.clients-5.2.0-ice35-b101.mac_Java6.zip | 5c05fd76 (SHA1) |

- OMERO.web is part of the server package, so individual users do not need to install it locally.
- Full instructions for installing the client are on the Help website: [Getting Started with OMERO insight Version 5.2.0](#)

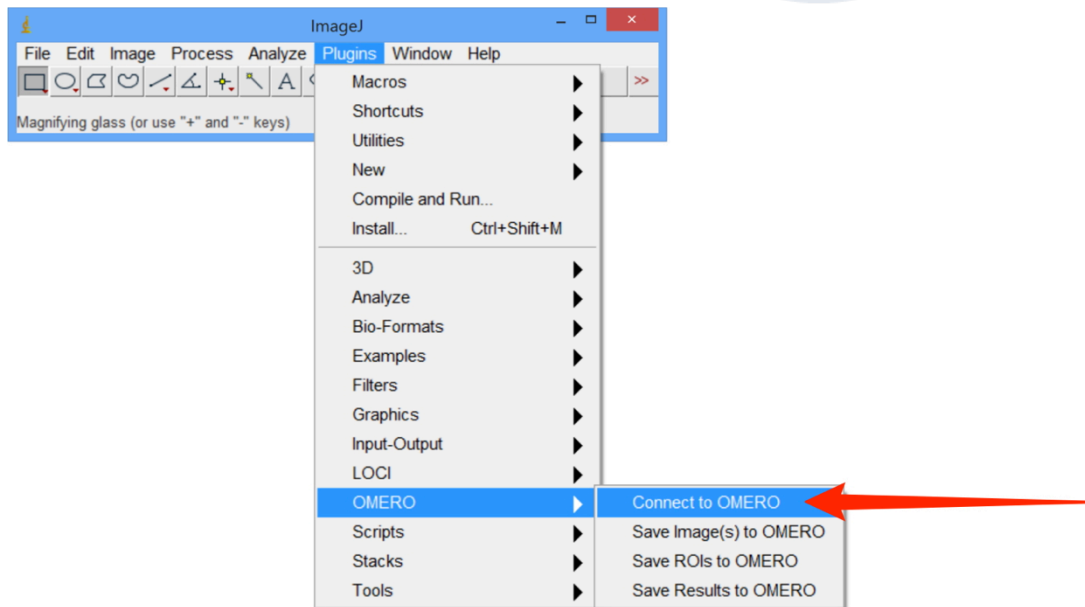
OMERO plugin downloads

| Plugins | Size | File Name | Checksum |
|---------------|----------|---------------------------------------|-----------------|
| ImageJ / Fiji | 75.28 MB | OMERO.insight-ij-5.2.0-ice35-b101.zip | 4ac3f188 (SHA1) |
| Matlab | 21.14 MB | OMERO.matlab-5.1.0-ice35-b101.zip | 9f9dfeef (SHA1) |

- Instructions for downloading and installing the ImageJ plugin: [Using ImageJ with OMERO](#)

Save or move the .zip archive to the FIJI > plugins folder.

Accessing OMERO using ImageJ and FIJI



ImageJ/FIJI and OMERO



Hoechst_ND - n000001.tif (33.3%)

35.84x27.31 inches (2688x2048), 16-bit, 11MB

ROI Manager

- Add [t]
- Update
- Delete
- Rename...
- Measure
- Deselect
- Properties...
- Flatten [F]
- More >
- Show All
- Show Labels

| Label | Area | Mean | StdDev | Mode | Min | Max | X | Y | |
|-------|--------------------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|---|
| 1 | Hoechst_ND - n000001.tif | 4.35556E-2 | 2.30549E3 | 4.88510E2 | 2.51500E3 | 1.51600E3 | 3.45700E3 | 1.97412E0 | 2 |
| 2 | Hoechst_ND - n000001.tif | 1.21600E-1 | 3.33402E3 | 8.96595E2 | 4.09500E3 | 1.51600E3 | 4.09500E3 | 2.36955E0 | 2 |
| 3 | Hoechst_ND - n000001.tif | 3.64444E-2 | 2.49201E3 | 5.14563E2 | 1.71900E3 | 1.55400E3 | 3.54300E3 | 9.42481E0 | 2 |



Hoechst_ND - n000001.tif

Viewing Options

- Normal
- Max Intensity
- Split Channel
- Quality: Normal
- Zoom (%): 43
- Line Plot
- Rendering Details
- Channels - Edit
- Grayscale
- Rendering Settings
- Interpolate
- Current Image
- Z: 1/1 T: 1/1
- Scale bar
- Image Information
- Image Link
- ROI Count: 1476
- Show ROIs | Hide
- < Prev Next >

| ID | Z | T | Text | Preview | Visibility |
|------|-----------|---|------|---------|-------------------------------------|
| 6051 | 0001-0009 | | | | <input checked="" type="checkbox"/> |
| 6052 | 0002-0017 | | | | <input checked="" type="checkbox"/> |
| 6053 | 0003-0011 | | | | <input checked="" type="checkbox"/> |
| 6054 | 0004-0010 | | | | <input checked="" type="checkbox"/> |
| 6055 | 0005-0008 | | | | <input checked="" type="checkbox"/> |
| 6056 | 0006-0016 | | | | <input checked="" type="checkbox"/> |
| 6057 | 0007-0025 | | | | <input checked="" type="checkbox"/> |
| 6058 | 0008-0018 | | | | <input checked="" type="checkbox"/> |
| 6059 | 0009-0023 | | | | <input checked="" type="checkbox"/> |
| 6060 | 0010-0025 | | | | <input checked="" type="checkbox"/> |
| 6061 | 0011-0037 | | | | <input checked="" type="checkbox"/> |
| 6062 | 0012-0033 | | | | <input checked="" type="checkbox"/> |
| 6063 | 0013-0034 | | | | <input checked="" type="checkbox"/> |
| 6064 | 0014-0027 | | | | <input checked="" type="checkbox"/> |
| 6065 | 0015-0030 | | | | <input checked="" type="checkbox"/> |
| 6066 | 0016-0032 | | | | <input checked="" type="checkbox"/> |
| 6067 | 0017-0023 | | | | <input checked="" type="checkbox"/> |
| 6068 | 0018-0037 | | | | <input checked="" type="checkbox"/> |
| 6069 | 0019-0036 | | | | <input checked="" type="checkbox"/> |
| 6070 | 0020-0038 | | | | <input checked="" type="checkbox"/> |
| 6071 | 0021-0042 | | | | <input checked="" type="checkbox"/> |
| 6072 | 0022-0044 | | | | <input checked="" type="checkbox"/> |

Timepoints

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Some useful links

- OMERO Downloads:

- <http://downloads.openmicroscopy.org/omero/>

- OMERO Help Pages:

- <http://help.openmicroscopy.org/>

- OMERO Forums:

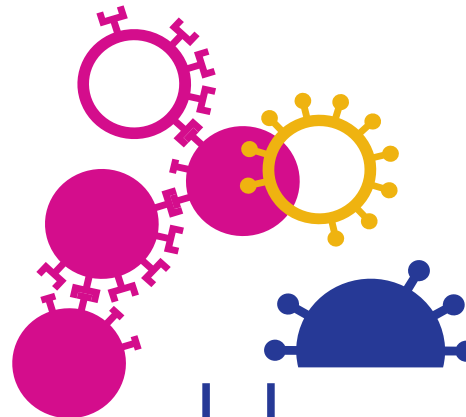
- <https://www.openmicroscopy.org/community/viewforum.php?f=3>

Thank to Funders



HORIZON 2020

wellcometrust



bbsrc

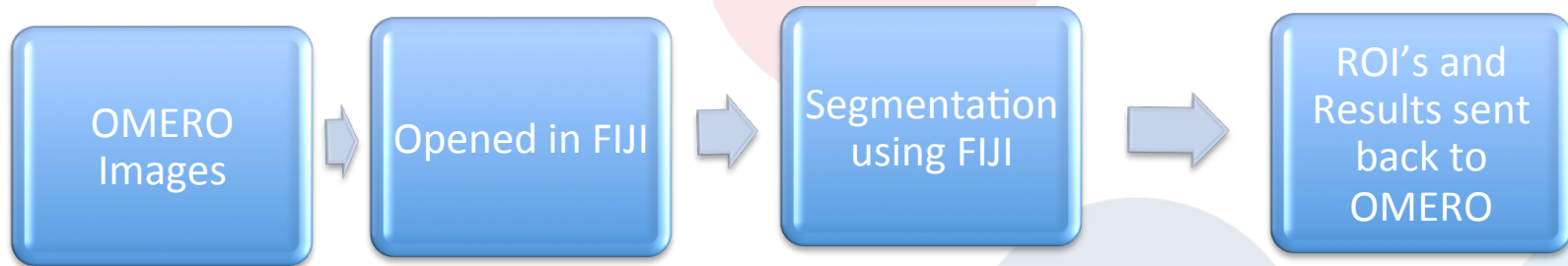
biotechnology and biological sciences
research council

OME Consortium

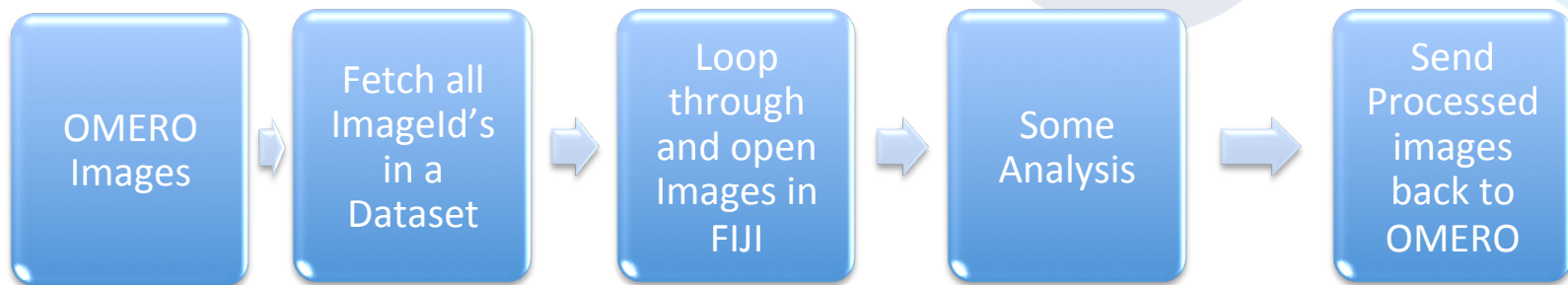
- Dundee – Jason Swedlow, Colin Blackburn, Jean-Marie Burel, Mark Carroll, Gus Ferguson, Helen Flynn, Kenny Gillen, Roger Leigh, Simon Li, Dominik Lindner, Josh Moore, Will Moore, Balaji Ramalingam, Aleksandra Tarkowska, Petr Walczysko
- University of Wisconsin, Madison (LOCI) - Kevin Eliceiri, Curtis Rueden, Mark Hiner
- UT Southwestern – Gaudenz Danuser, Sebastian Besson
- Oxford – Ilan Davis, Douglas Russell
- CRS4 - Gianuigi Zanetti, Gianmauro Cucurru, Simone Leo, Luca Lianas
- Edinburgh – Richard Baldock, Bill Hill, Jianguo Rao
- Carnegie-Mellon – Robert Murphy, BK Cho, Ivan Cao-Berg
- Imperial – Paul French, Chris Dunsby, Ian Munro, Yuriy Alexandrov
- NIA, NIH – Ilya Goldberg, Chris Coletta
- Pasteur – Spencer Shorte, Sebastien Simard, Anne Danckaert
- EBI – Gerard Kleywegt, Ardan Patwardhan, Ingvar Lagerstedt
- Glencoe Software – Chris Allan, Joshua Ballanco, Andreas Knab, Melissa Linkert, Chris MacLeod, Josh Moore, Emil Rozbicki, Liza Unson, Rebecca Walker, Wilma Woudenberg

FIJI-OMERO Workflow Outline

- Setup OMEROIJ Plugin
- Manual Workflow:

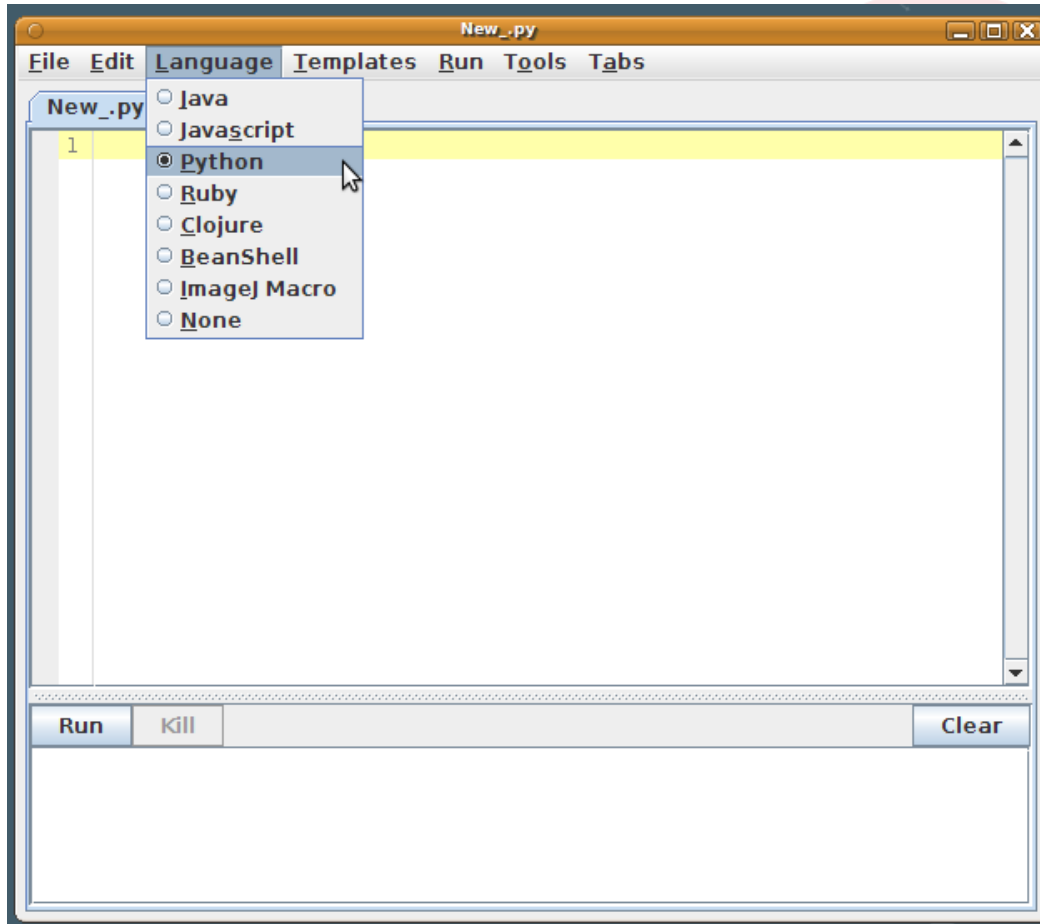


- Batch Analysis using FIJI scripts:

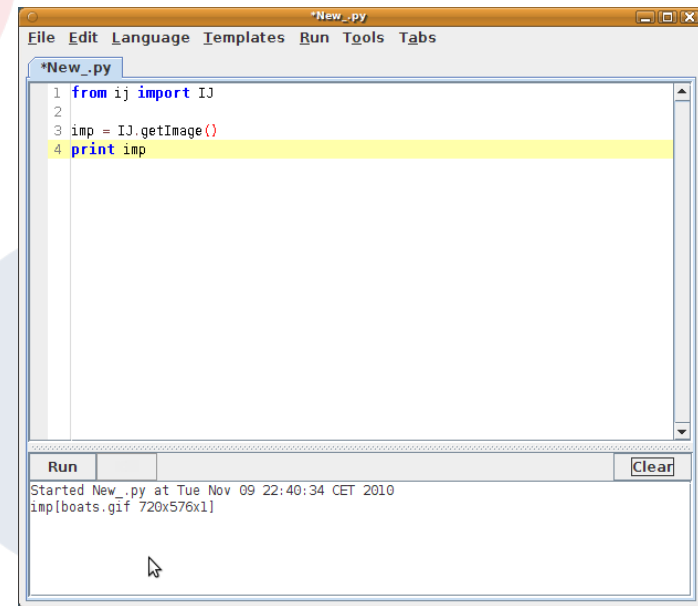


FIJI Scripts

FIJI : Script Editor



FIJI : Grabbing an open Image



OMERO-FIJI Scripts : Accessory Methods

```
def omeroConnect():
```

```
# Omero Connect with credentials and simpleLogger
cred = LoginCredentials()
cred.getServer().setHostname(HOST)
cred.getServer().setPort(PORT)
cred.getUser().setUsername(USERNAME)
cred.getUser().setPassword(PASSWORD)
simpleLogger = SimpleLogger()
gateway = Gateway(simpleLogger)
gateway.connect(cred)
return gateway
```

```
# List all ImageId's under a Project/Dataset
```

```
def getImageIds(gateway, datasetId):
```

```
browse = gateway.getFacility(BrowseFacility)
user = gateway.getLoggedInUser()
ctx = SecurityContext(user.getGroupId())
ids = ArrayList(1)
val = Long(datasetId)
ids.add(val)
images = browse.getImagesForDatasets(ctx, ids)
j = images.iterator()
imageIds = []
while j.hasNext():
    image = j.next()
    imageIds.append(String.valueOf(image.getId()))
return imageIds
```

```
def openImagePlus(HOST, USERNAME, PASSWORD, groupId, imageId):
```

```
options = ""
options += "location=[OMERO] open=[omero:server="
options += HOST
options += "\nuser="
options += USERNAME
options += "\npass="
options += PASSWORD
options += "\ngroupID="
options += groupId
options += "\niid="
options += imageId
options += "]"
options += " windowless=true "
```

```
print options
from ij import IJ
```

```
IJ.runPlugin("loci.plugins.LociImporter", options);
```

```
def uploadImage(path, gateway):
```

```
user = gateway.getLoggedInUser()
ctx = SecurityContext(user.getGroupId())
sessionKey = gateway.getSessionId(user)
```

```
config = ImportConfig()
```

```
config.email.set("")
config.sendFiles.set('true')
config.sendReport.set('false')
config.contOnError.set('false')
config.debug.set('false')
config.hostname.set(HOST)
config.sessionKey.set(sessionKey)
config.targetClass.set("omero.model.Dataset")
config.targetId.set(datasetId)
```

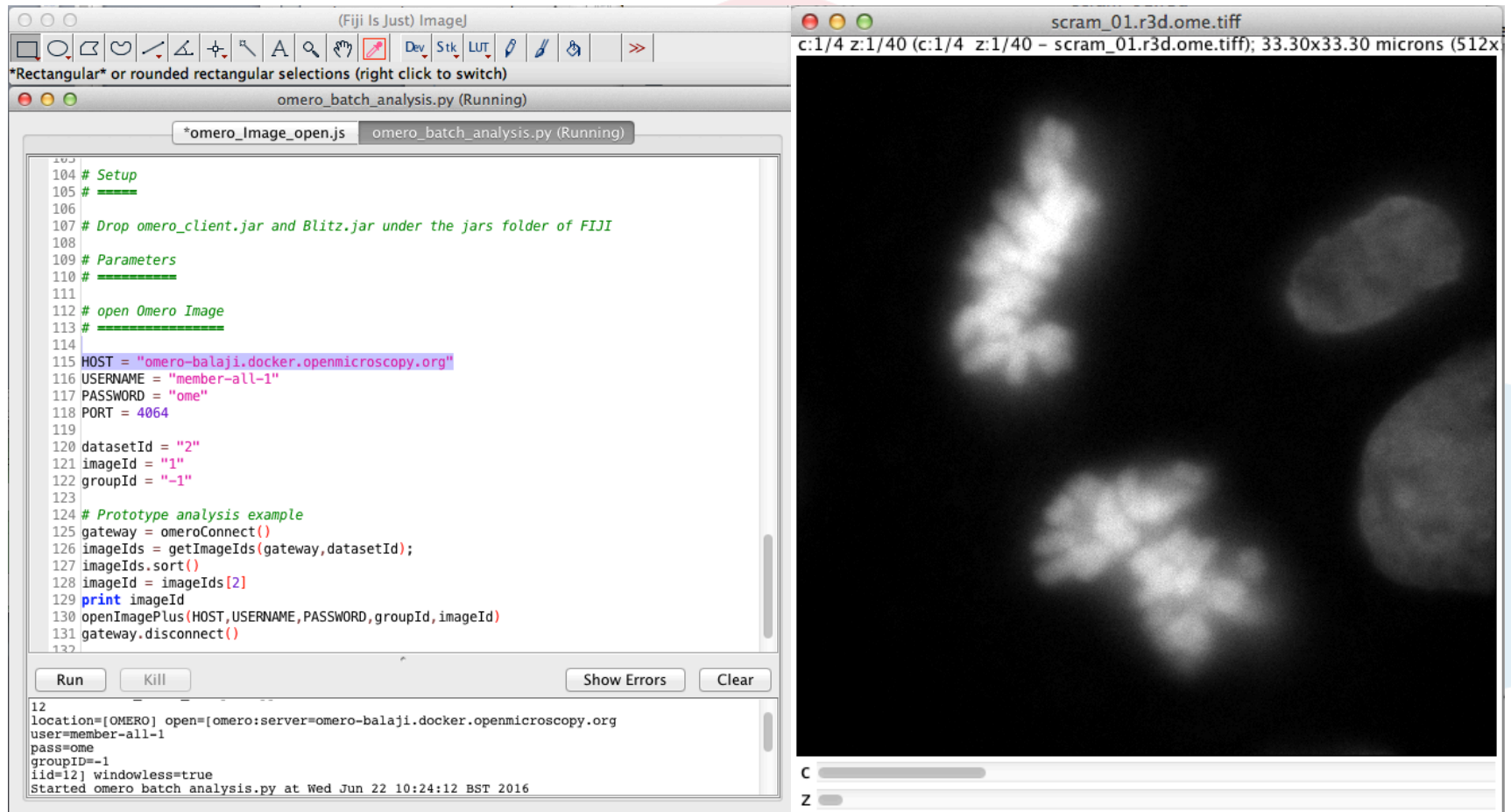
```
loci.common.DebugTools.enableLogging("DEBUG")
```

```
store = config.createStore()
reader = OMEROWrapper(config)
```

```
library = ImportLibrary(store, reader)
errorHandler = ErrorHandler(config)
```

```
library.addObserver(LoggingImportMonitor())
candidates = ImportCandidates(reader, path, errorHandler)
reader.setMetadataOptions(DefaultMetadataOptions(MetadataLevel.ALL))
success = library.importCandidates(config, candidates)
return success
```

FIJI Scripts : Client Side (DEMO)



The screenshot displays the FIJI (Fiji Is Just) ImageJ interface. On the left, a script window titled "omero_batch_analysis.py (Running)" is open, showing a Python script. The script includes comments for setup, parameters, and a prototype analysis example. The parameters section defines the Omero server host, username, password, port, dataset ID, image ID, and group ID. The prototype analysis example uses the omeroConnect() method to connect to the server, retrieve image IDs, and open an image plus metadata.

```
104 # Setup
105 # =====
106
107 # Drop omero_client.jar and Blitz.jar under the jars folder of FIJI
108
109 # Parameters
110 # =====
111
112 # open Omero Image
113 # =====
114
115 HOST = "omero-balaji.docker.openmicroscopy.org"
116 USERNAME = "member-all-1"
117 PASSWORD = "ome"
118 PORT = 4064
119
120 datasetId = "2"
121 imageId = "1"
122 groupId = "-1"
123
124 # Prototype analysis example
125 gateway = omeroConnect()
126 imageIds = getImageIds(gateway, datasetId);
127 imageIds.sort()
128 imageId = imageIds[2]
129 print imageId
130 openImagePlus(HOST, USERNAME, PASSWORD, groupId, imageId)
131 gateway.disconnect()
132
```

Below the script editor are buttons for "Run", "Kill", "Show Errors", and "Clear". The output window at the bottom shows the execution details:

```
12
location=[OMERO] open=[omero:server=omero-balaji.docker.openmicroscopy.org
user=member-all-1
pass=ome
groupID=-1
iid=12] windowless=true
Started omero batch analysis.py at Wed Jun 22 10:24:12 BST 2016
```

On the right, an image window titled "scram_01.r3d.ome.tiff" displays a grayscale microscopy image of biological cells. The image dimensions are 33.30x33.30 microns (512x512 pixels). The image shows several bright, irregularly shaped structures against a dark background.