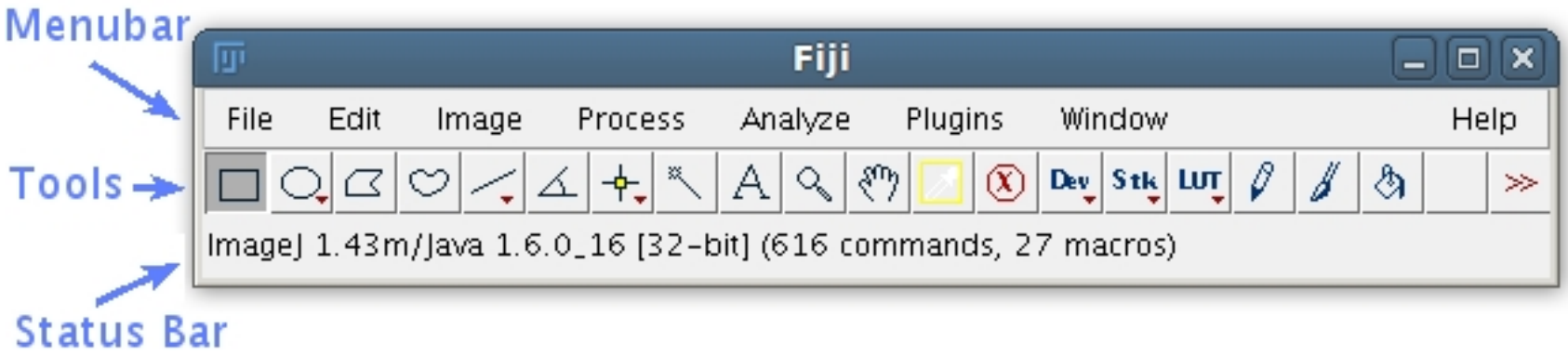
Outline

- 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

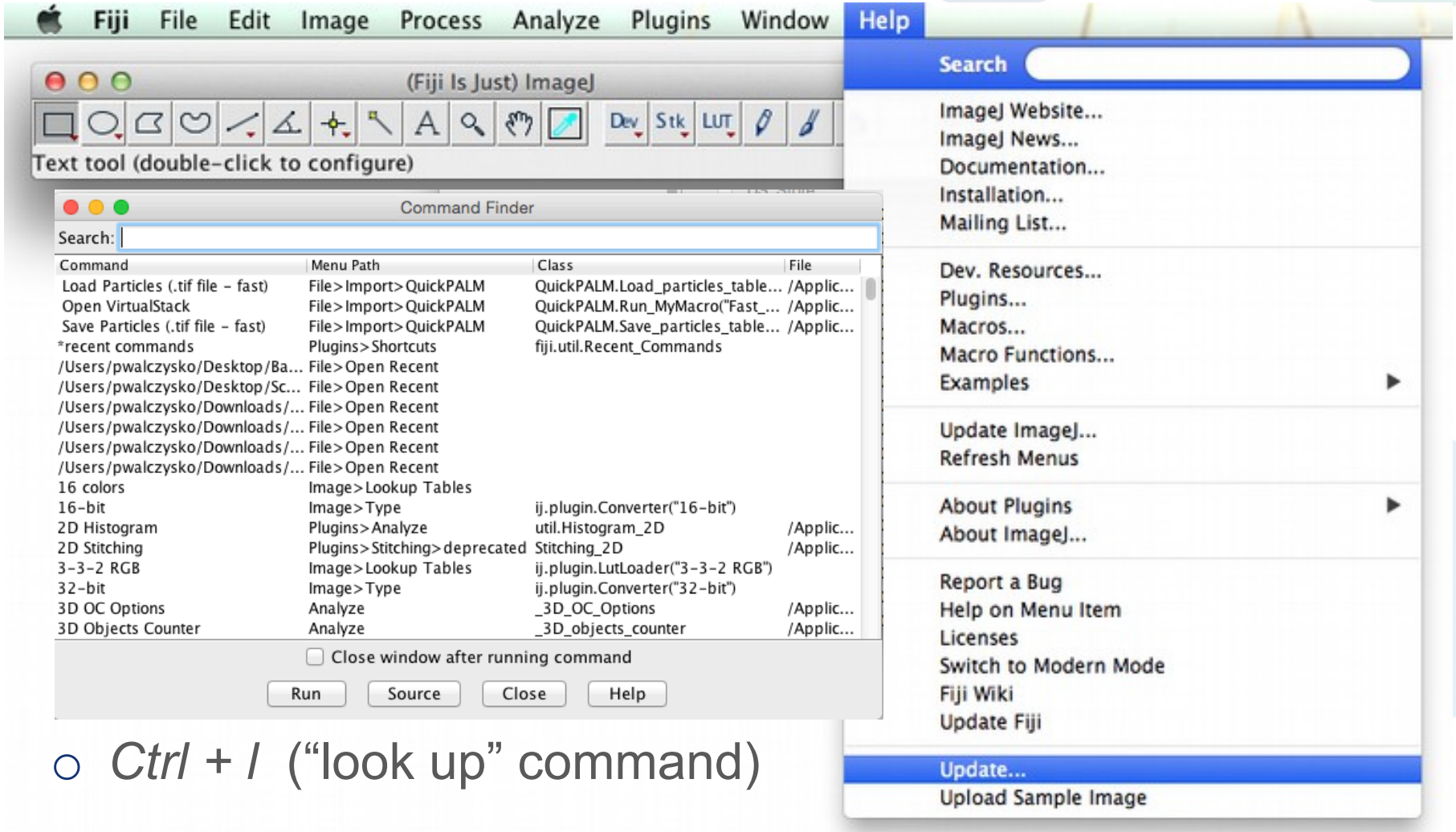
The main ImageJ window



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)

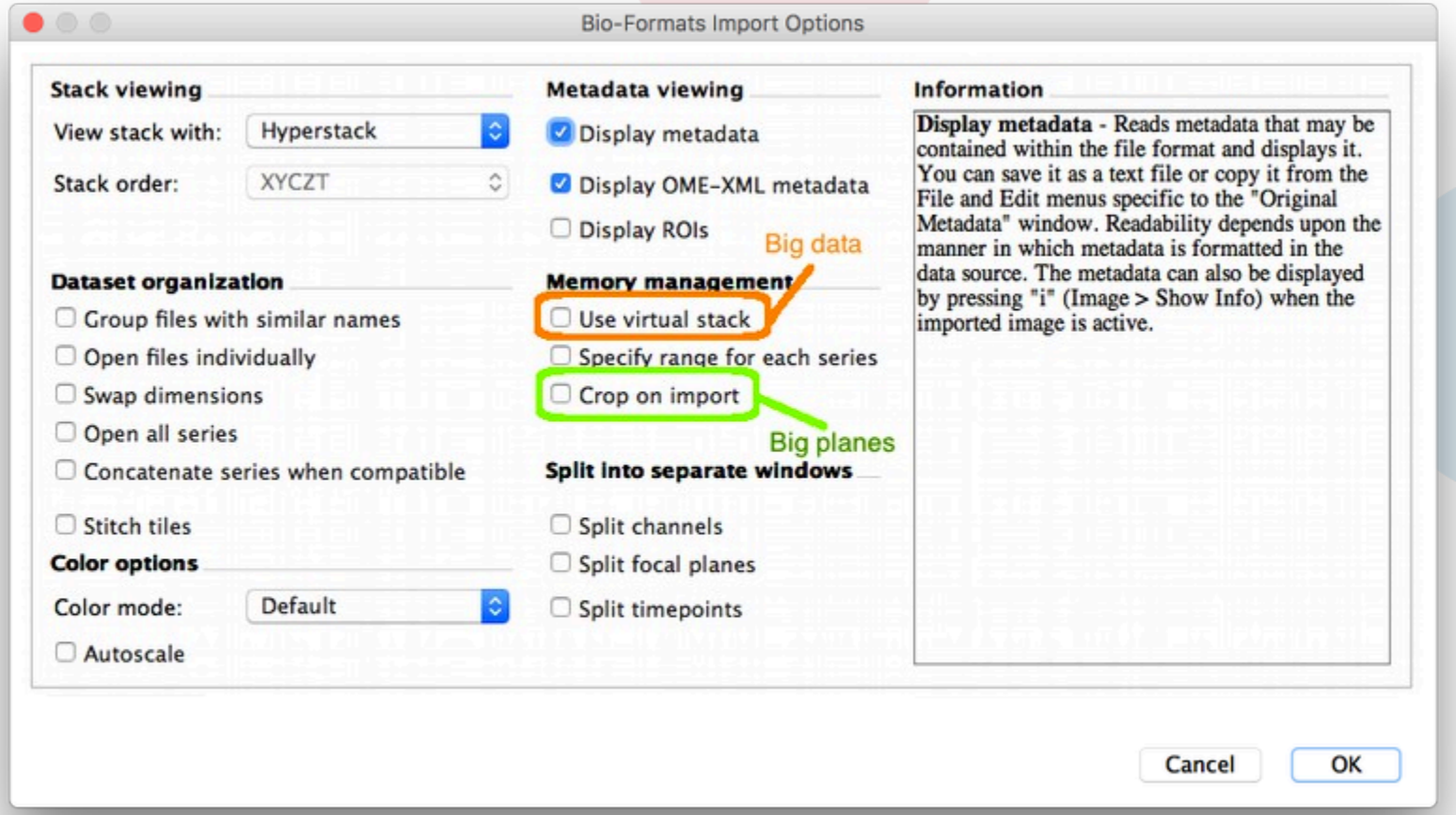
Help: finding commands & updates



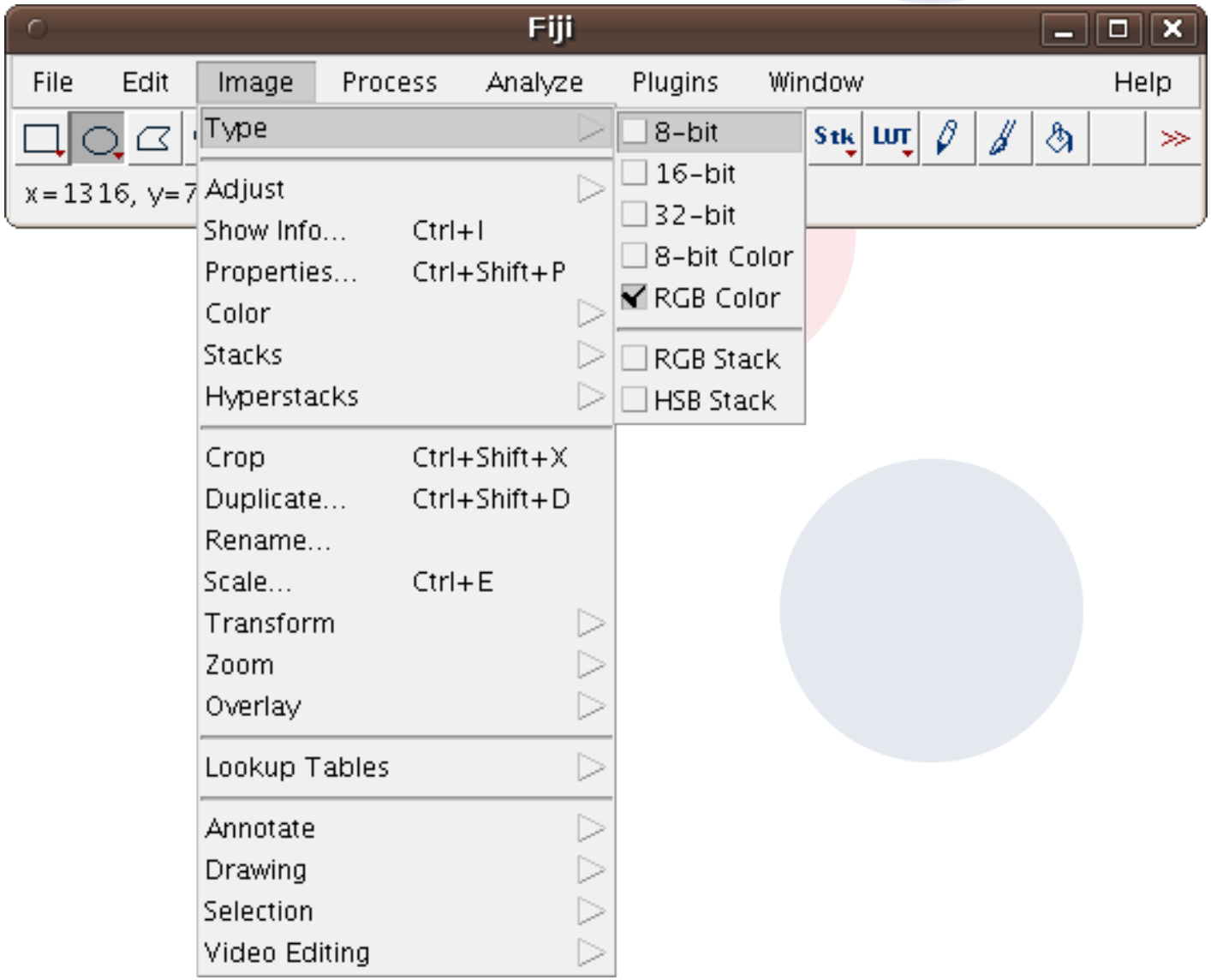
- *Ctrl* + *l* (“look up” command)
- or *Help* ▸ *Search* ▸ Type into the box

Opening data (and Bio-Formats)

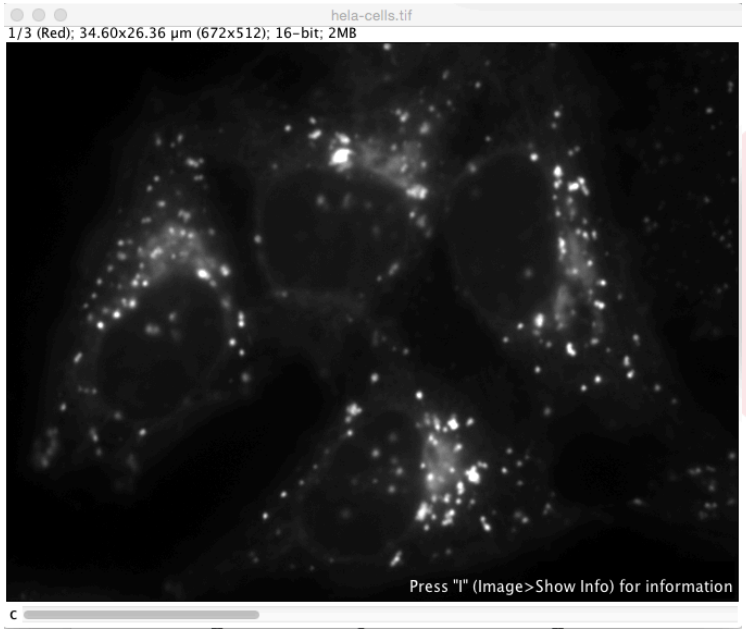
- Drag and Drop
- File > Open
- File > Import > Bio-Formats
- Plugins > Bio-Formats > Bio-Formats Importer

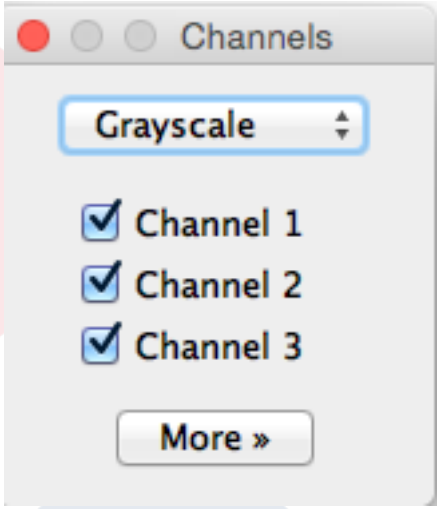


Pixel Types


Fiji
File
Edit
Image
Process
Analyze
Plugins
Window
Help
x=1316, y=7
Type
Adjust
Show Info... Ctrl+I
Properties... Ctrl+Shift+P
Color
Stacks
Hyperstacks
Crop Ctrl+Shift+X
Duplicate... Ctrl+Shift+D
Rename...
Scale... Ctrl+E
Transform
Zoom
Overlay
Lookup Tables
Annotate
Drawing
Selection
Video Editing
8-bit
16-bit
32-bit
8-bit Color
RGB Color
RGB Stack
HSB Stack
Stk
LUT

Channels Tool

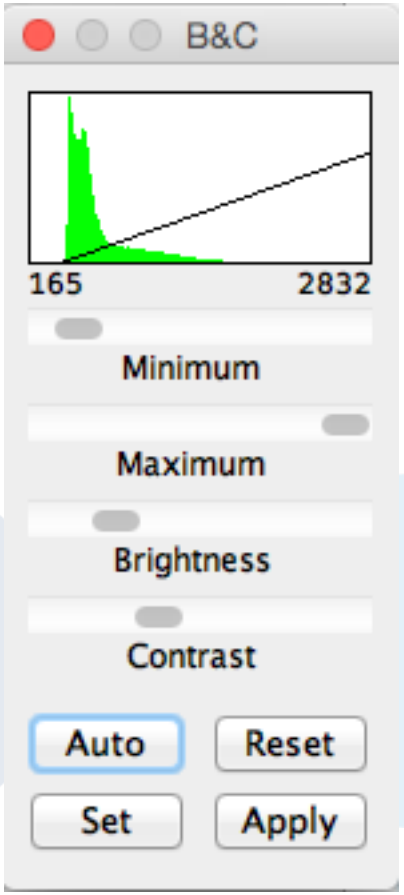




- Switch Channels on and off, adjust Brightness and Contrast
- *File* ▸ *Open Samples* ▸ *HeLa*
- *Image* ▸ *Color* ▸ *Channels Tool* (Shift + Z) ▸ *Grayscale*
- Switch to Color, then switch to Composite
- *More* ▸ *Properties* ▸ *Convert to RGB* for publication or output

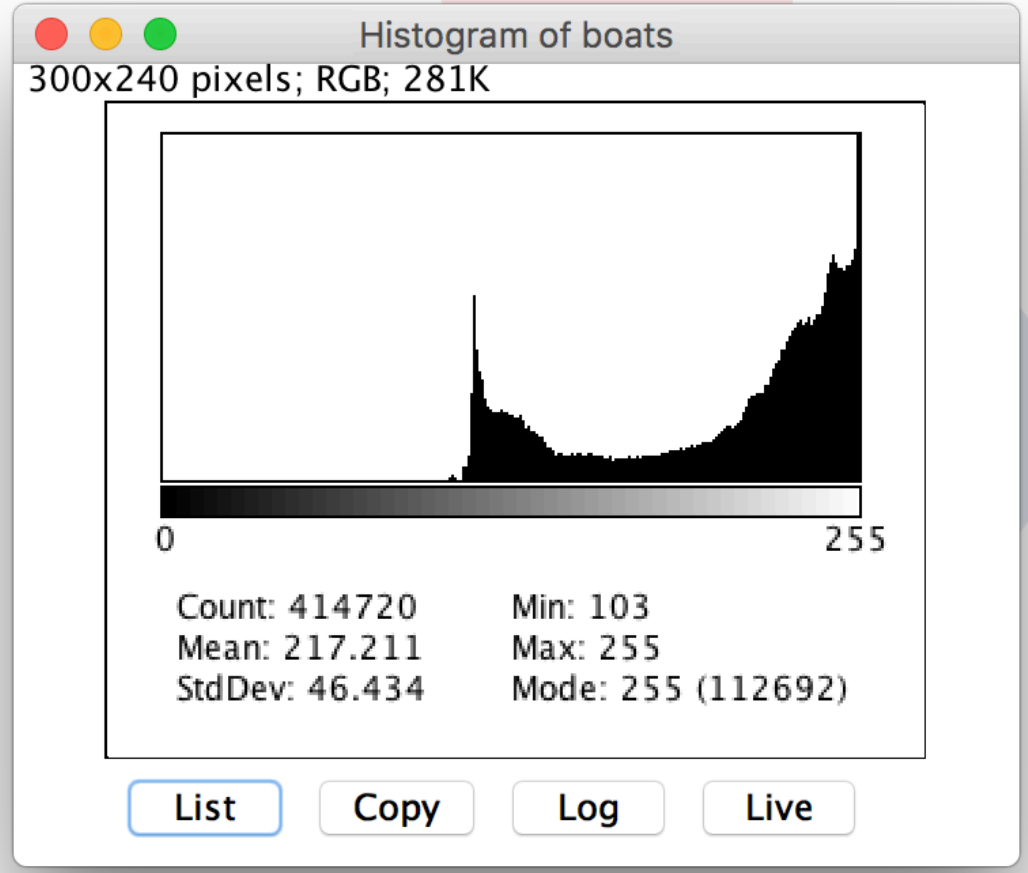
Adjust Brightness and Contrast

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



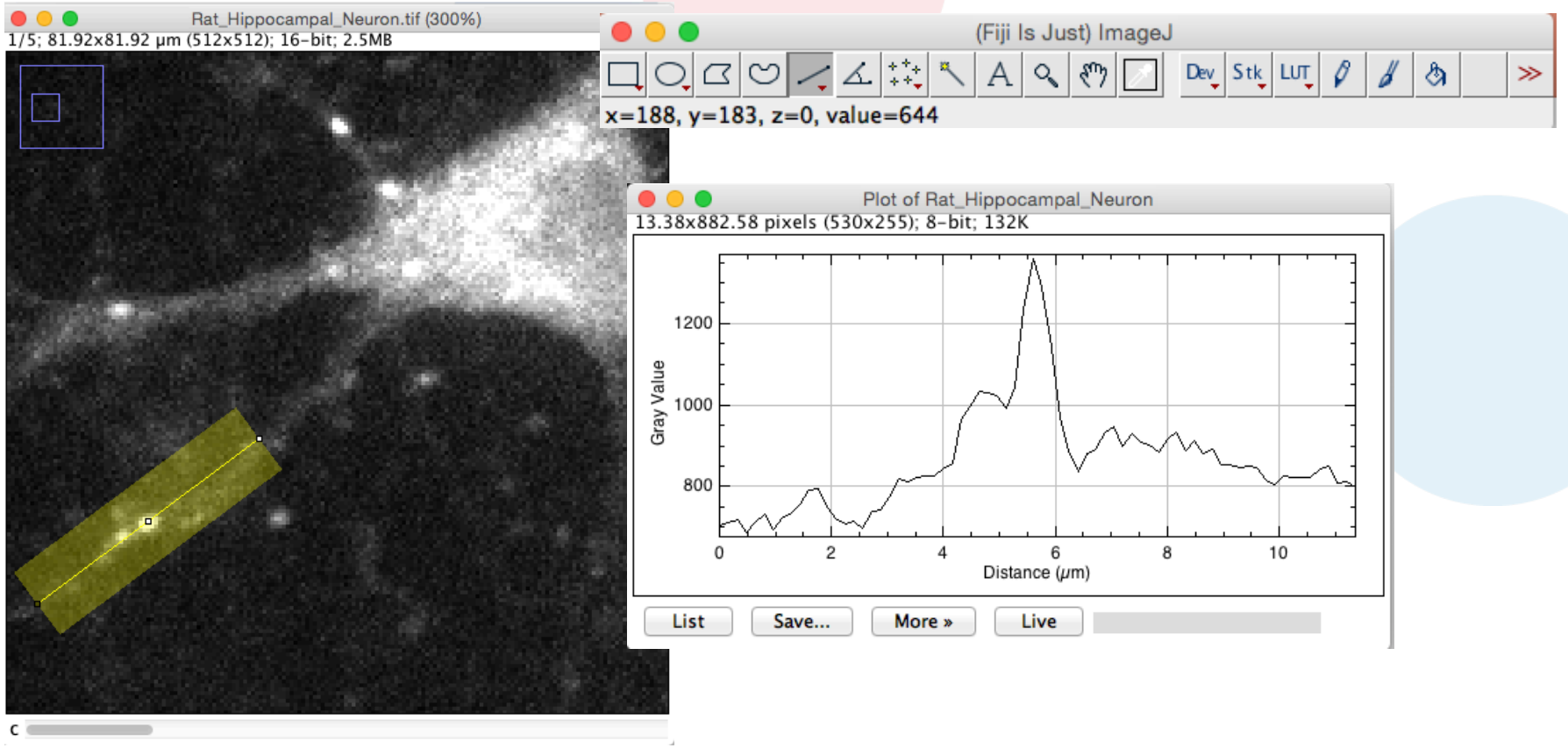
Histogram

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



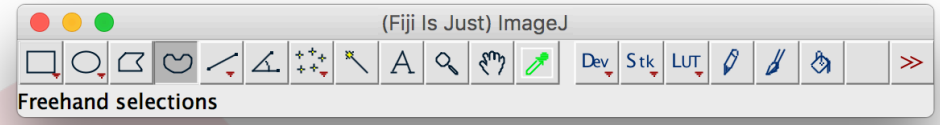
Profile Plots

- 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?

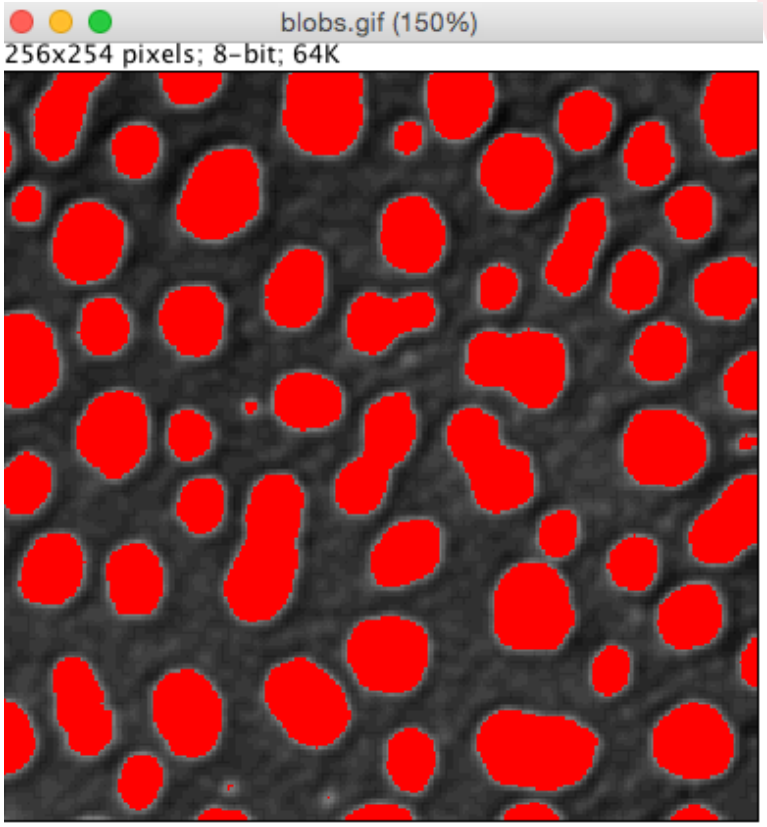


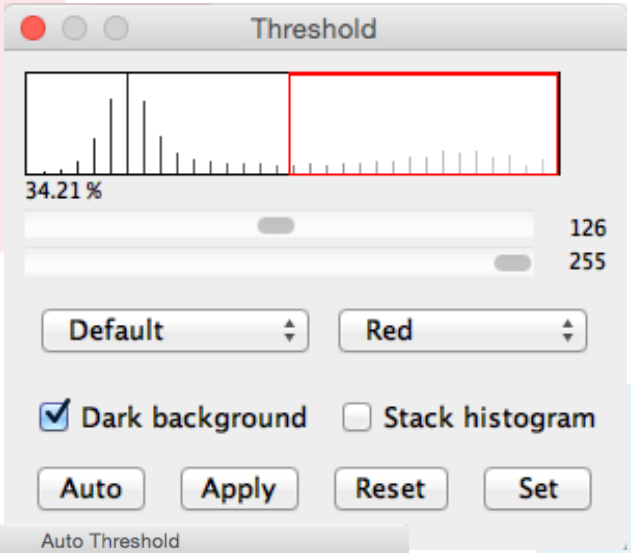
The ROI Manager: a “clipboard” for ROIs

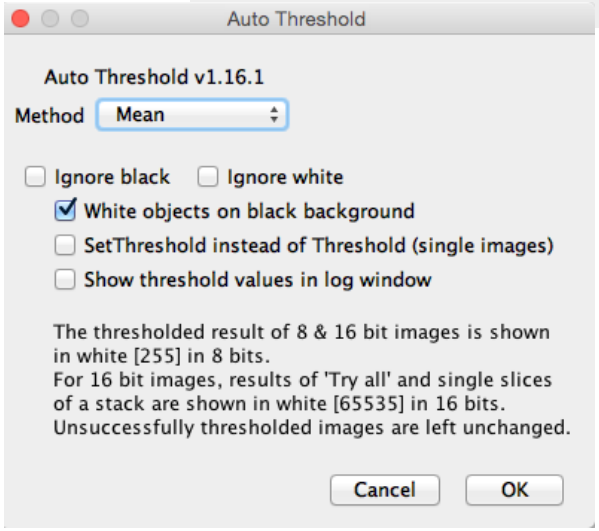
- Select the image with a ROI
- Press “*T*” key – **or** *Analyze* ▸ *Tools* ▸ *ROI Manager*
- Select other image
- Click ROI in manager – **or** *Edit* ▸ *Selection* ▸ *Restore Selection*

Segmentation (by intensity threshold)

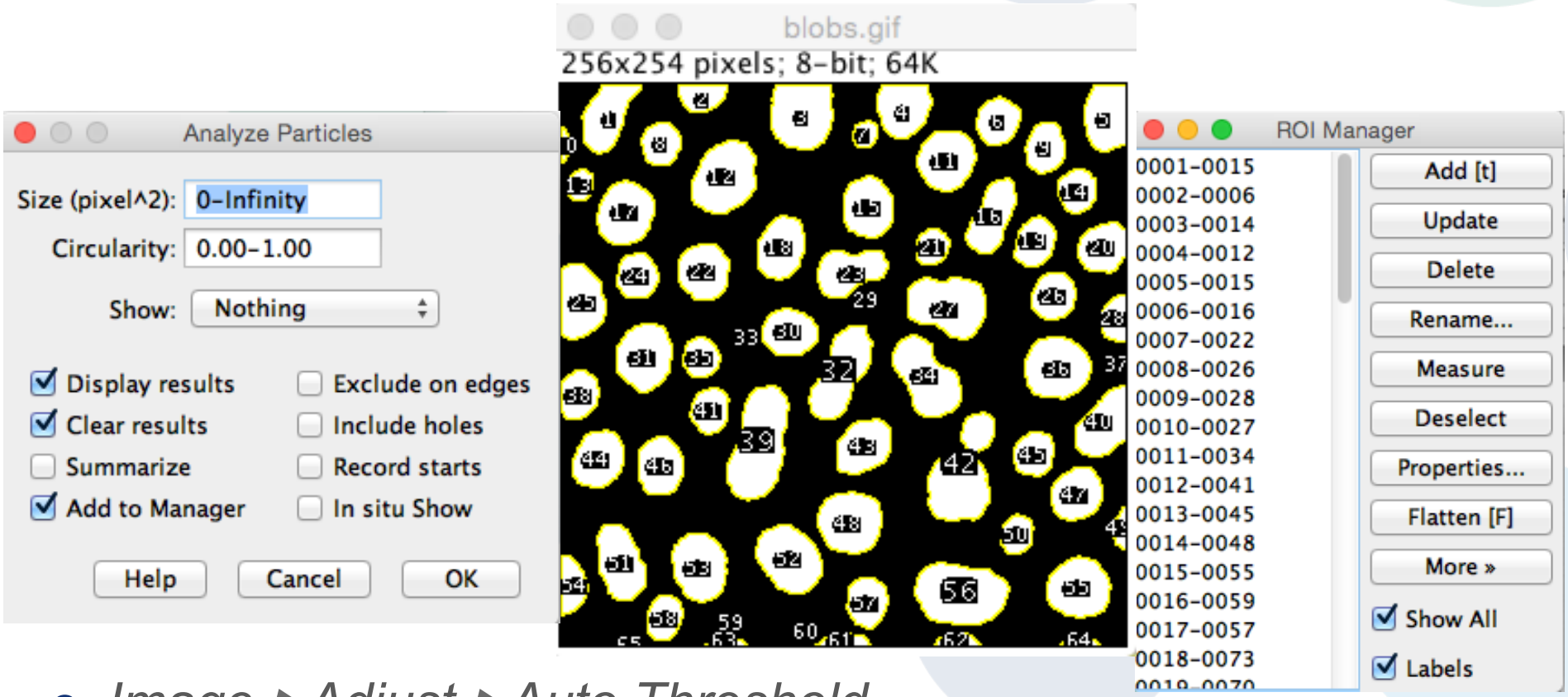
- *Image* ▸ *Adjust* ▸ *Threshold...* (Shift + T)
- *Image* ▸ *Adjust* ▸ *Auto Threshold...* (Shift + T)





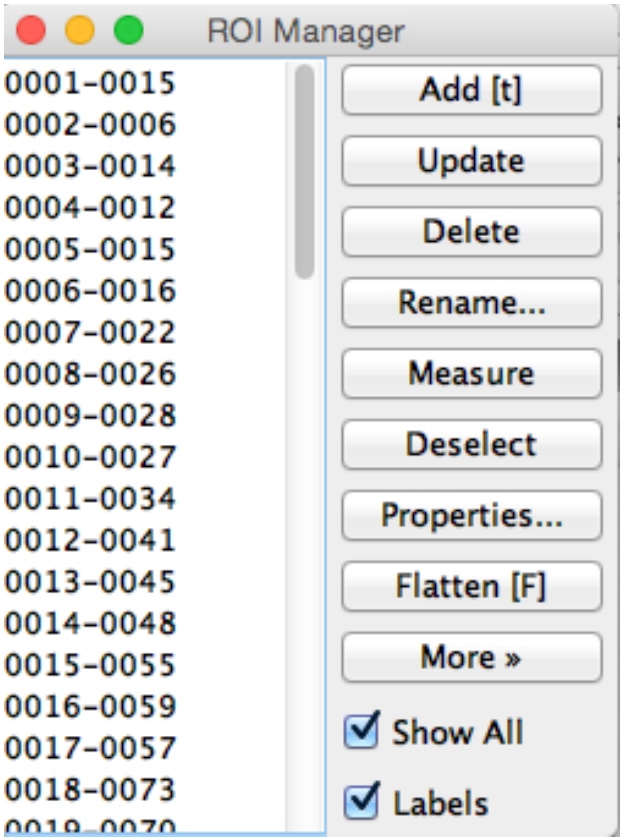


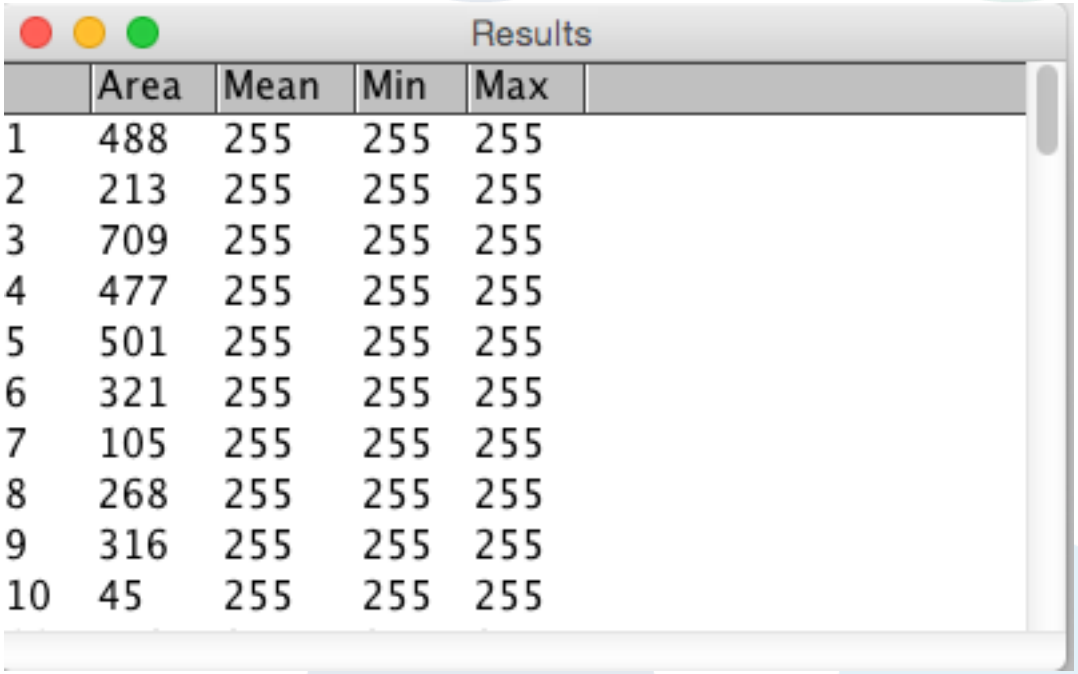
Identifying, labeling & measuring objects



- *Image* ▸ *Adjust* ▸ *Auto Threshold*
- *Analyze* ▸ ***Analyze Particles...***
  - *Display results* checkbox is checked -> Results table appears

Results table





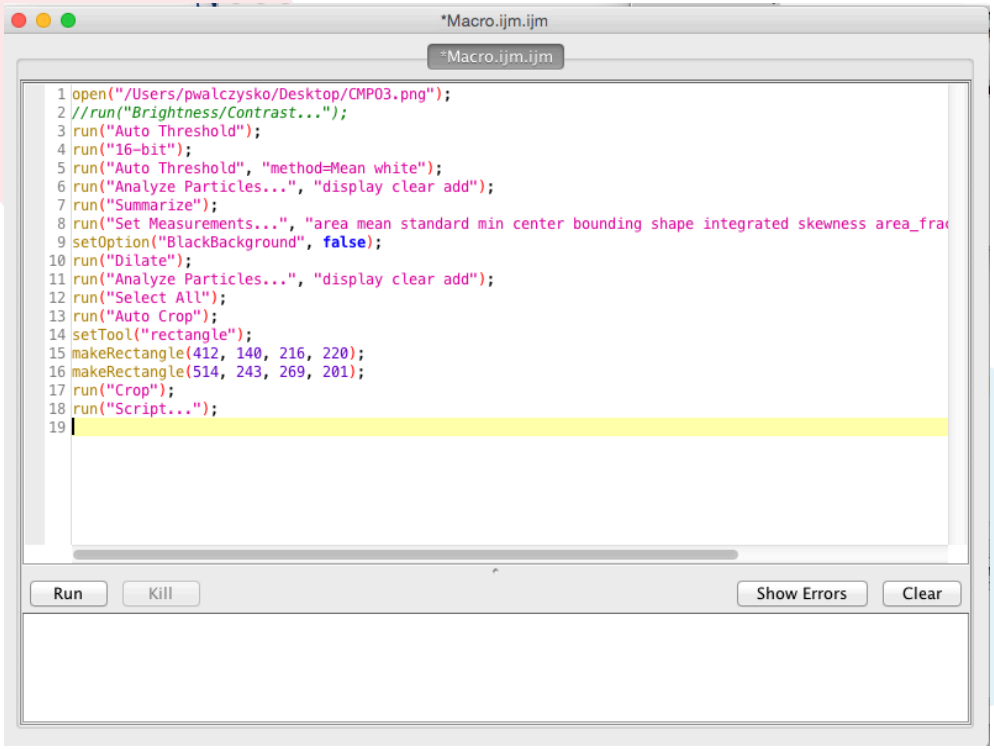
	Area	Mean	Min	Max
1	488	255	255	255
2	213	255	255	255
3	709	255	255	255
4	477	255	255	255
5	501	255	255	255
6	321	255	255	255
7	105	255	255	255
8	268	255	255	255
9	316	255	255	255
10	45	255	255	255

- 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:

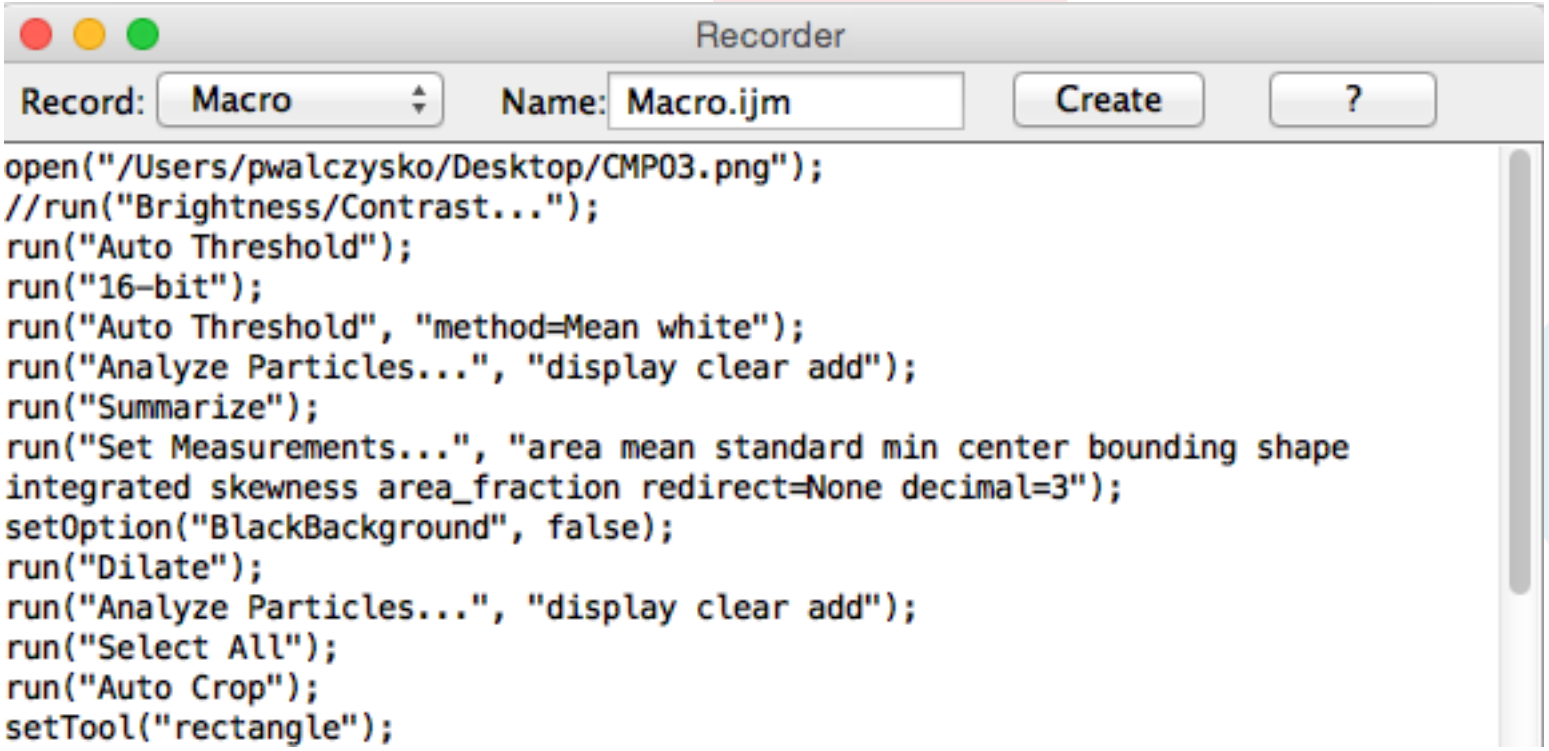
- Automate analysis
- Document your work
- Share workflows



the Fiji Script Editor

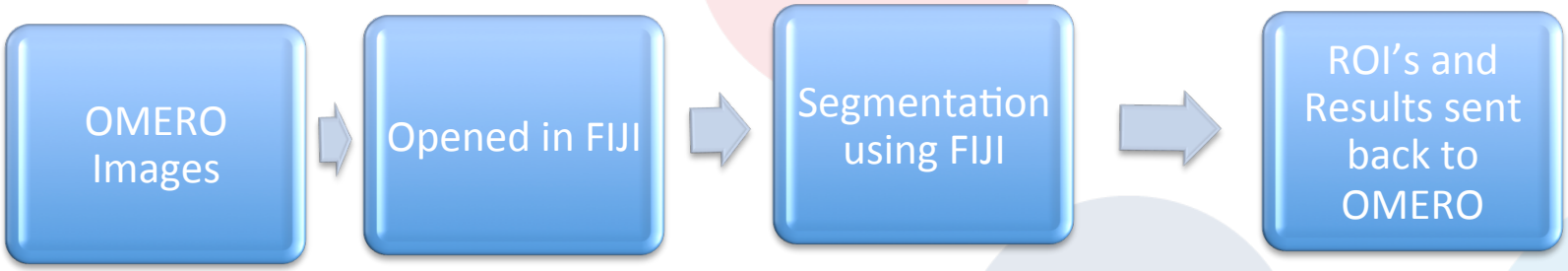
Macros are easy in ImageJ

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

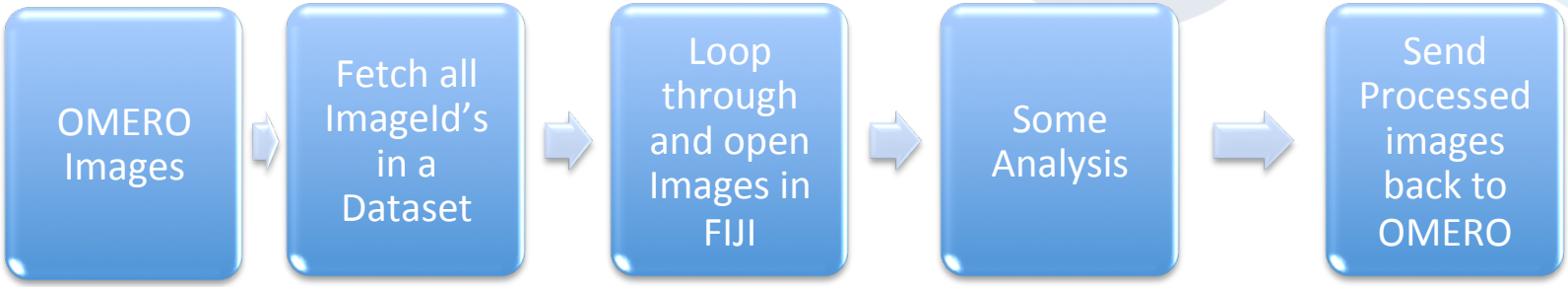


FIJI-OMERO Workflow Outline

- Setup OMEROIJ Plugin
- Manual Workflow:

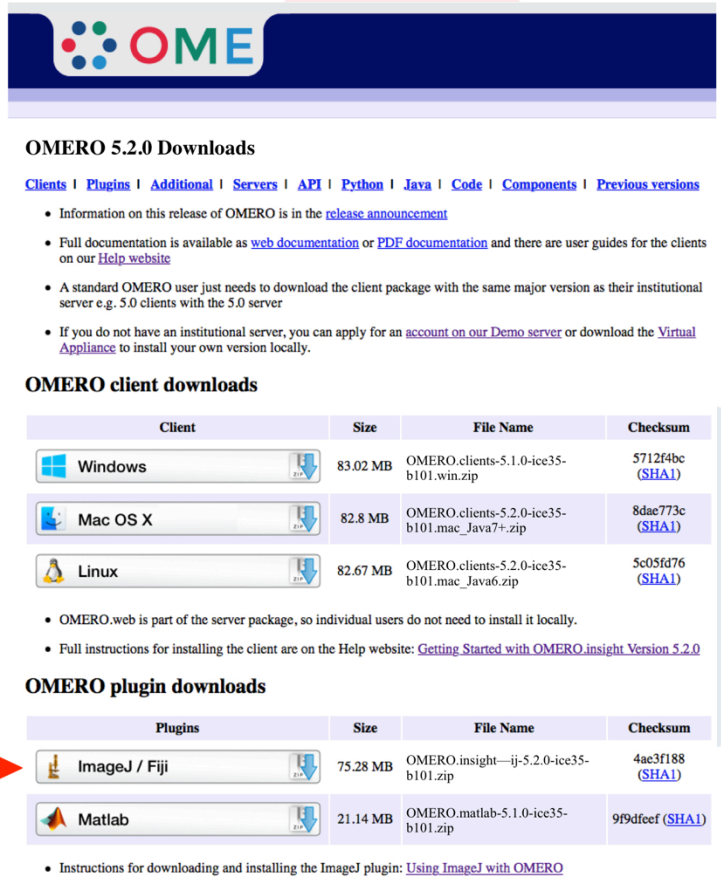


- Batch Analysis using FIJI scripts:



OMEROIJ Plugin : Setup

Dependency : OMERO IJ Plugin



OME

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

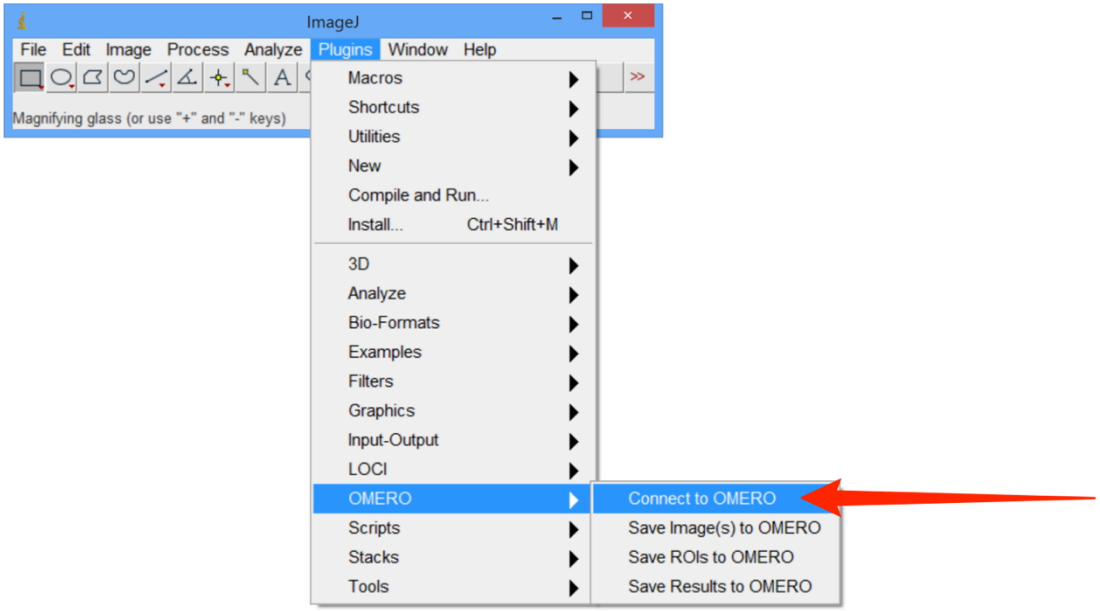
Plugins	Size	File Name	Checksum
ImageJ / Fiji	75.28 MB	OMERO.insight—ij-5.2.0-ice35-b101.zip	4ae3f188 (SHA1)
Matlab	21.14 MB	OMERO.matlab-5.1.0-ice35-b101.zip	9f9dfeef (SHA1)

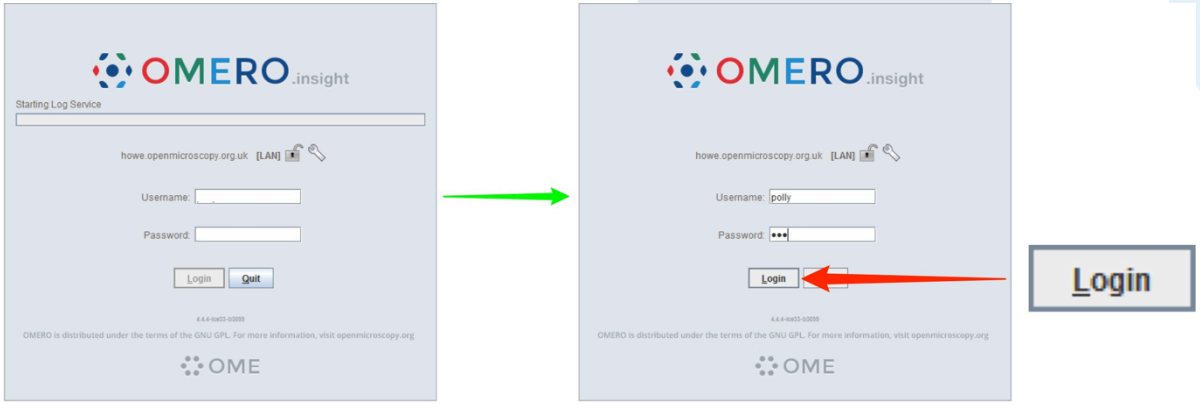
- Instructions for downloading and installing the ImageJ plugin: Using ImageJ with OMERO

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

http://help.openmicroscopy.org/imagej.html

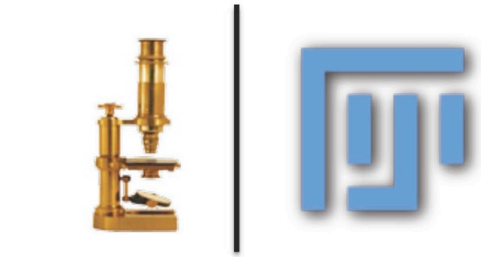
Accessing OMERO using ImageJ and FIJI

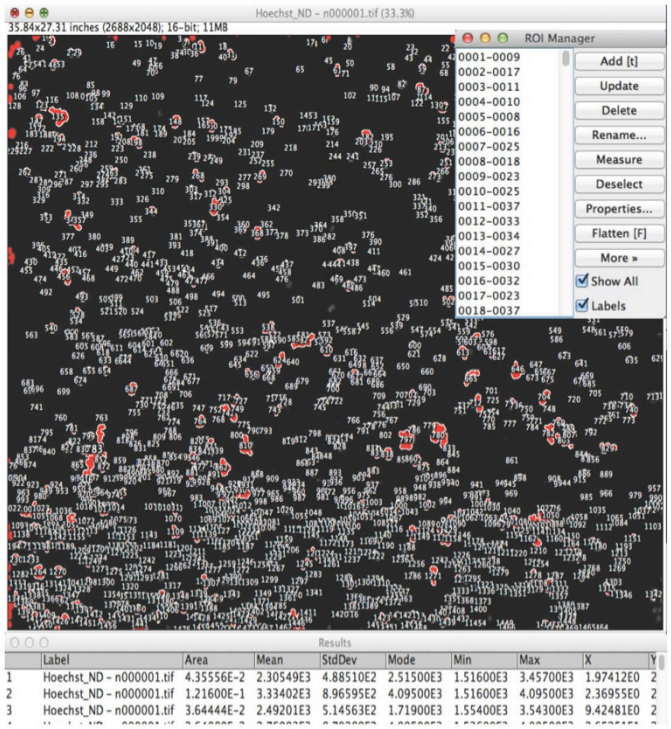


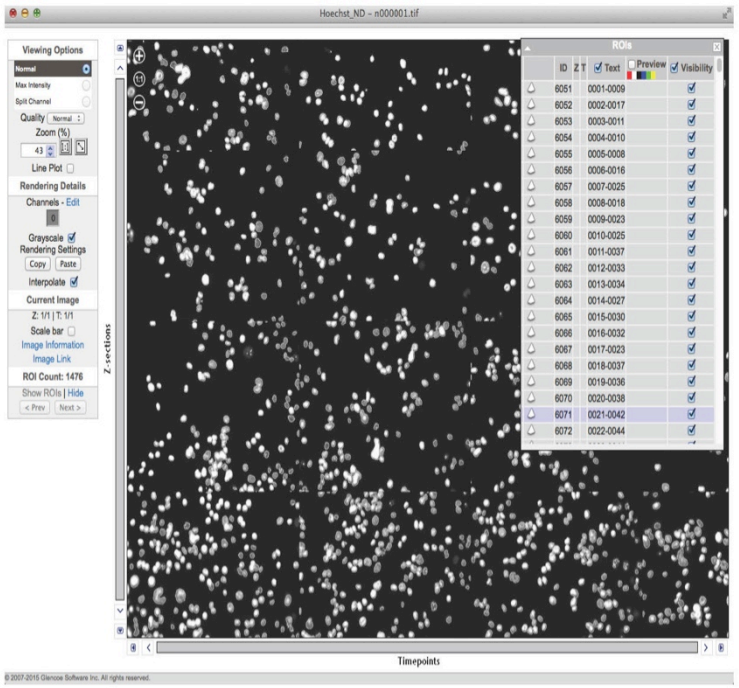


http://help.openmicroscopy.org/imagej.html

ImageJ/FIJI and OMERO







http://help.openmicroscopy.org/imagej.html

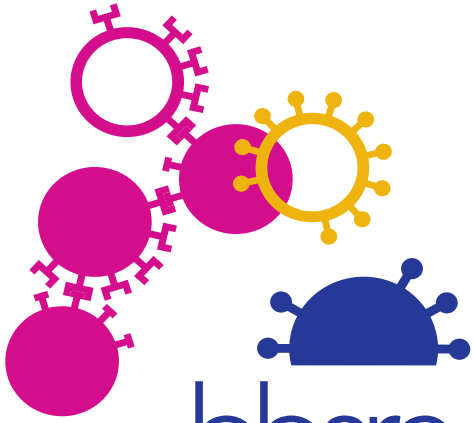
Some useful links

- 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

wellcome trust



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

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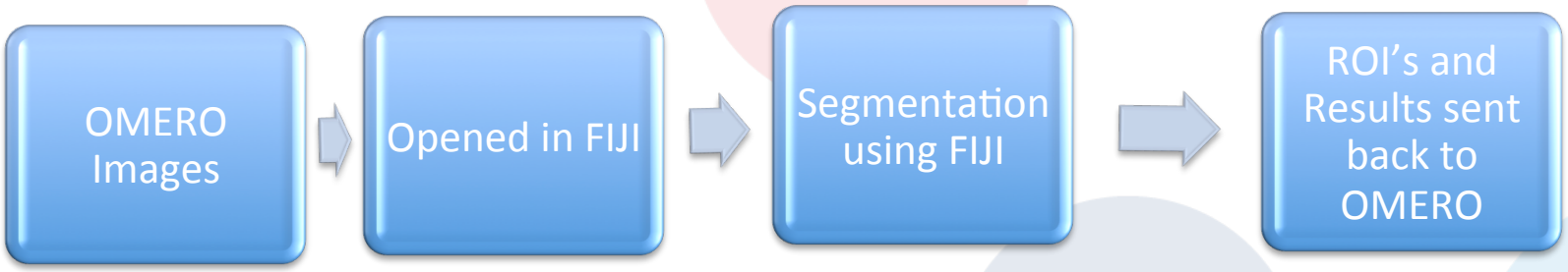
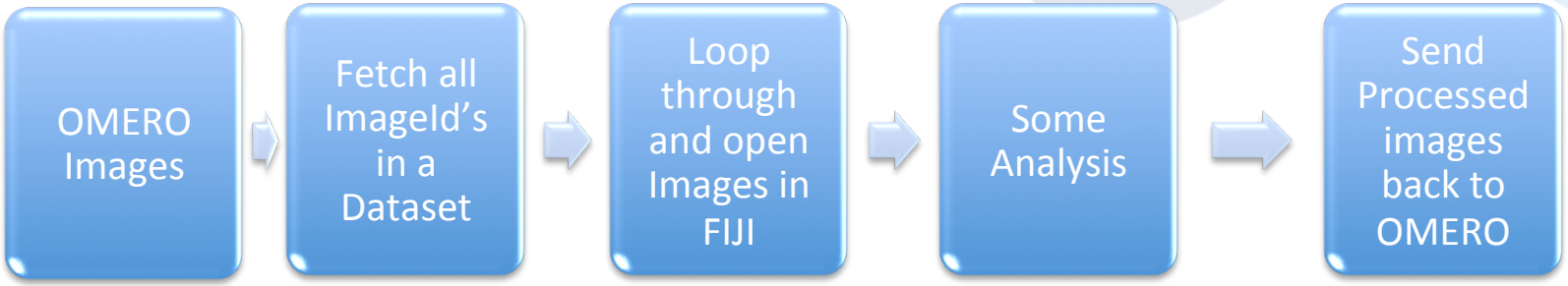
FIJI-OMERO Workflow Outline

- Setup OMEROIJ Plugin
- Manual Workflow:

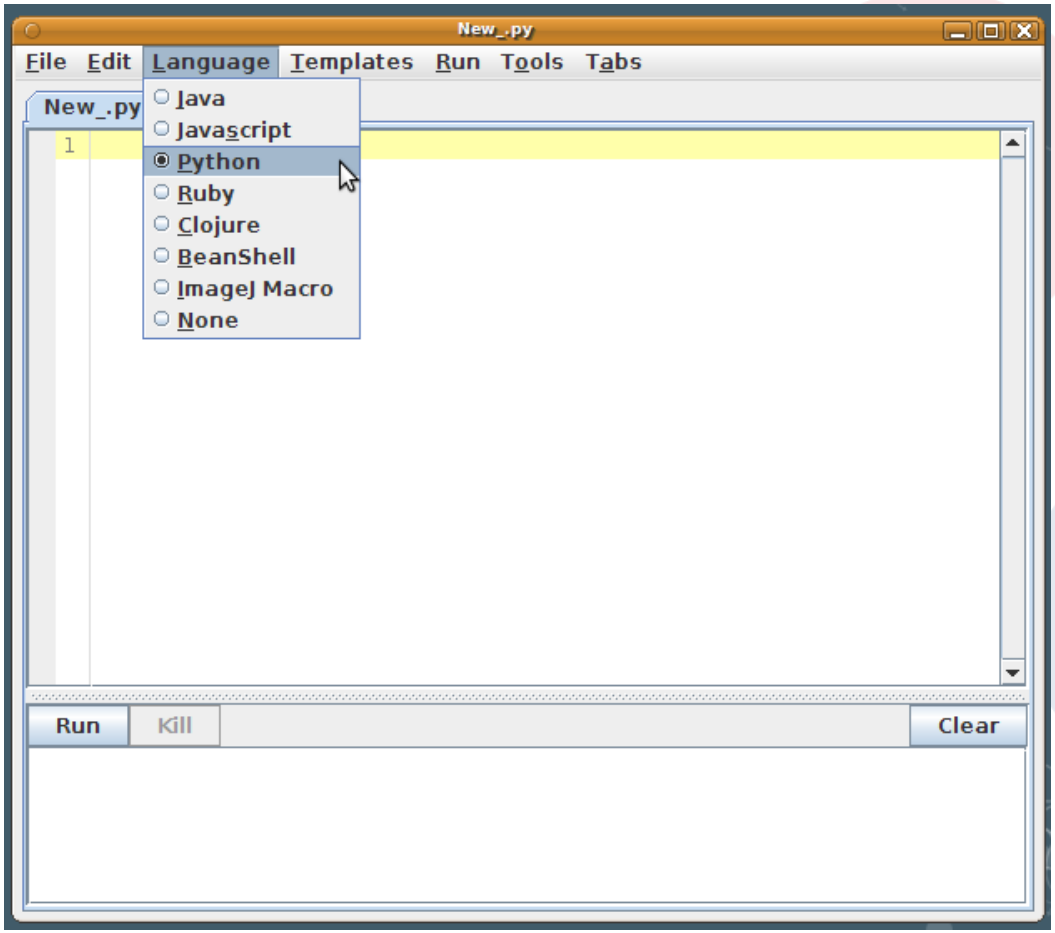


- Batch Analysis using FIJI scripts:

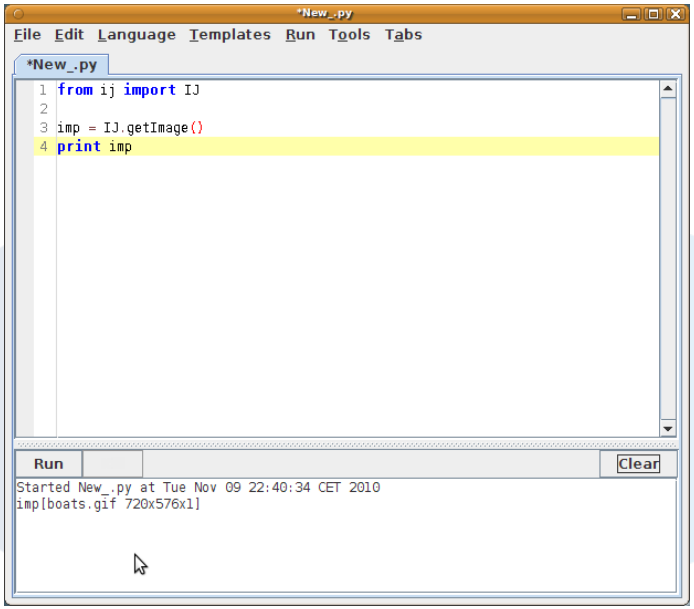


FIJI Scripts

FIJI : Script Editor



FIJI : Grabbing an open Image



https://www.ini.uzh.ch/~acardona/fiji-tutorial/

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)