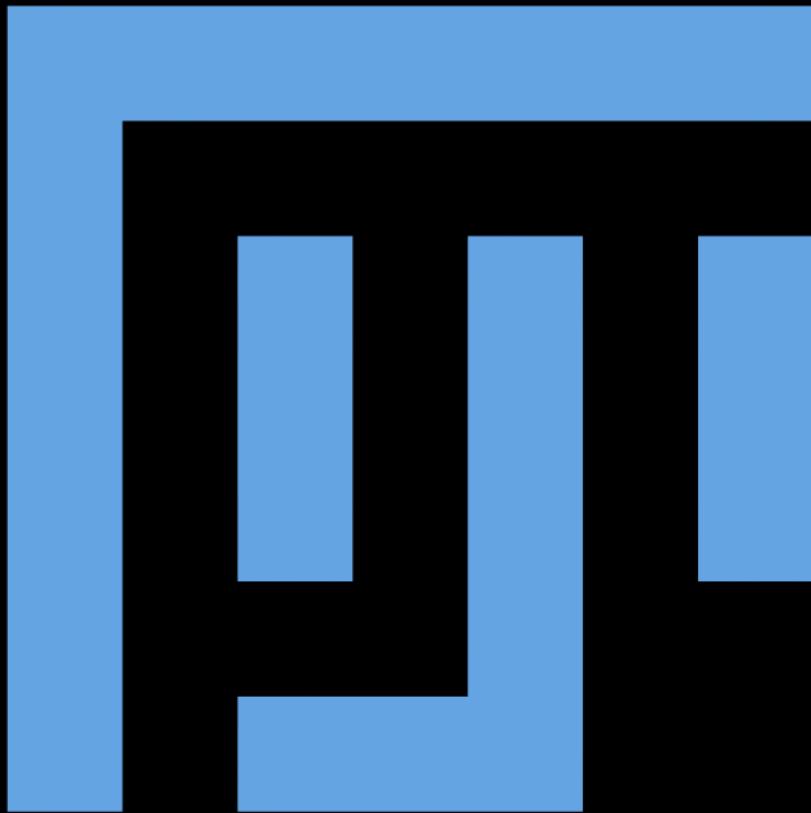
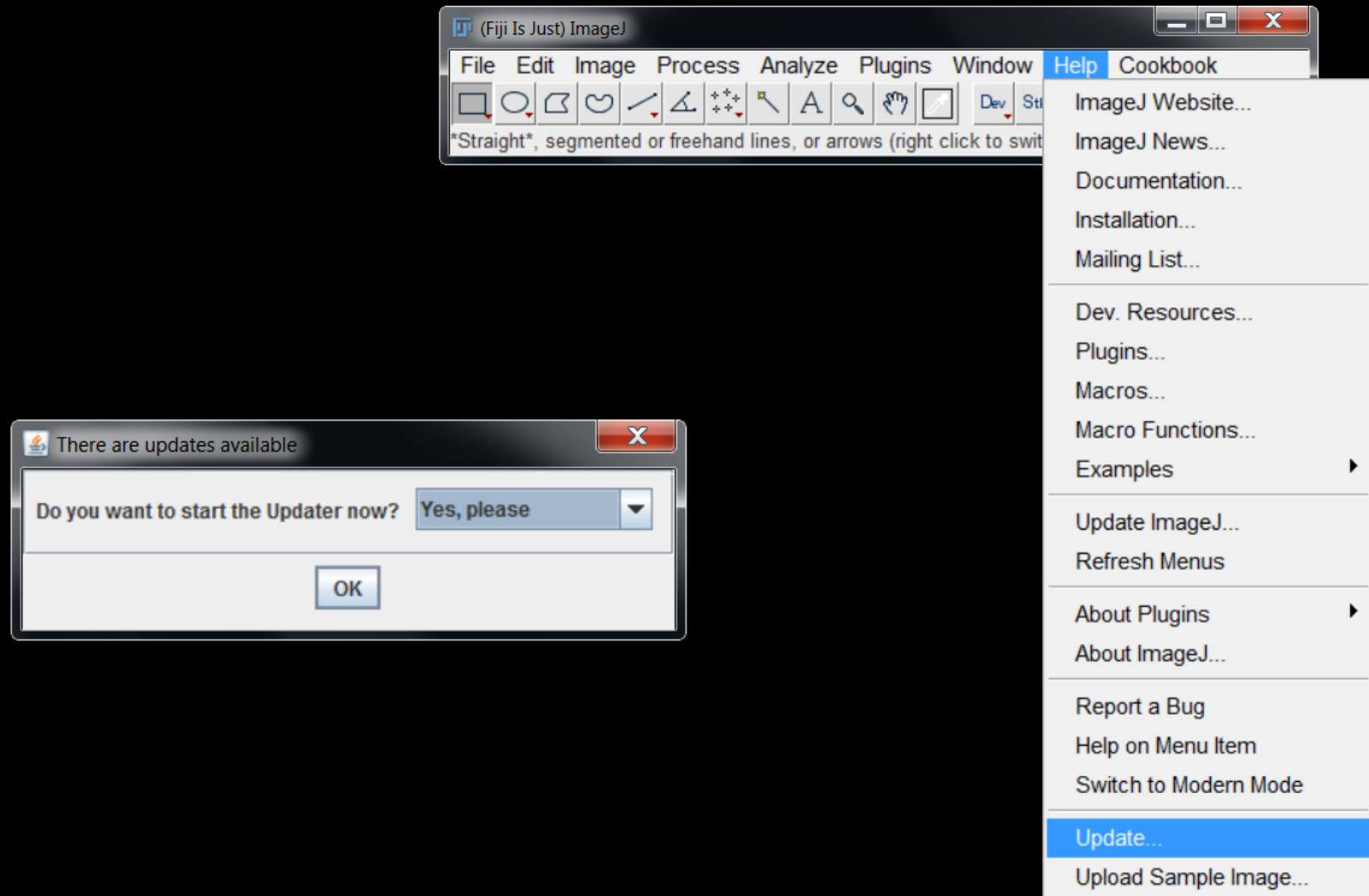


Introduction to Image Analysis with



PLEASE ENSURE FIJI IS INSTALLED CORRECTLY!



WHAT DO WE HOPE TO ACHIEVE?

The screenshot shows a website interface for 'THE FRANCIS CRICK INSTITUTE'. On the left, there's a sidebar with 'CrickNet' at the top, followed by 'PROFILE' and 'MY PINS'. The main content area has a search bar with placeholder text 'Search people, events, information' and a magnifying glass icon. Below the search bar are navigation links: 'SCIENCE', 'PEOPLE', 'OPERATIONS & BUILDING', and 'COMMUNITY', each with a dropdown arrow. Underneath these, there are links for 'HOME', 'COMMUNITY', and 'SEMINARS & EVENTS'. To the right of these links is a yellow button with the text 'Pin this' and a pin icon. In the center, there's a box for a seminar: '19 JAN' (date) and '14:00-17:00' (time). To the right of this box is the title 'Introduction to image analysis with FIJI'.

Specifically, the workshop will cover the following topics:

1. Opening images with Bioformats
2. Interpreting histograms
3. Basic segmentation
4. Filtering images
5. Intro to morphological quantification
6. Analysis of protein expression

- Basic morphological quantification of cells and spatial analysis of protein expression.

AIMS

- Introduction to image analysis concepts
- Gentle introduction to FIJI

FORMAT

- General overview
- Small chunks of theory/demonstration, each followed by a practical.
- Challenges
- Break for tea/coffee at 15:30
- Finish about 17:00

HOUSEKEEPING

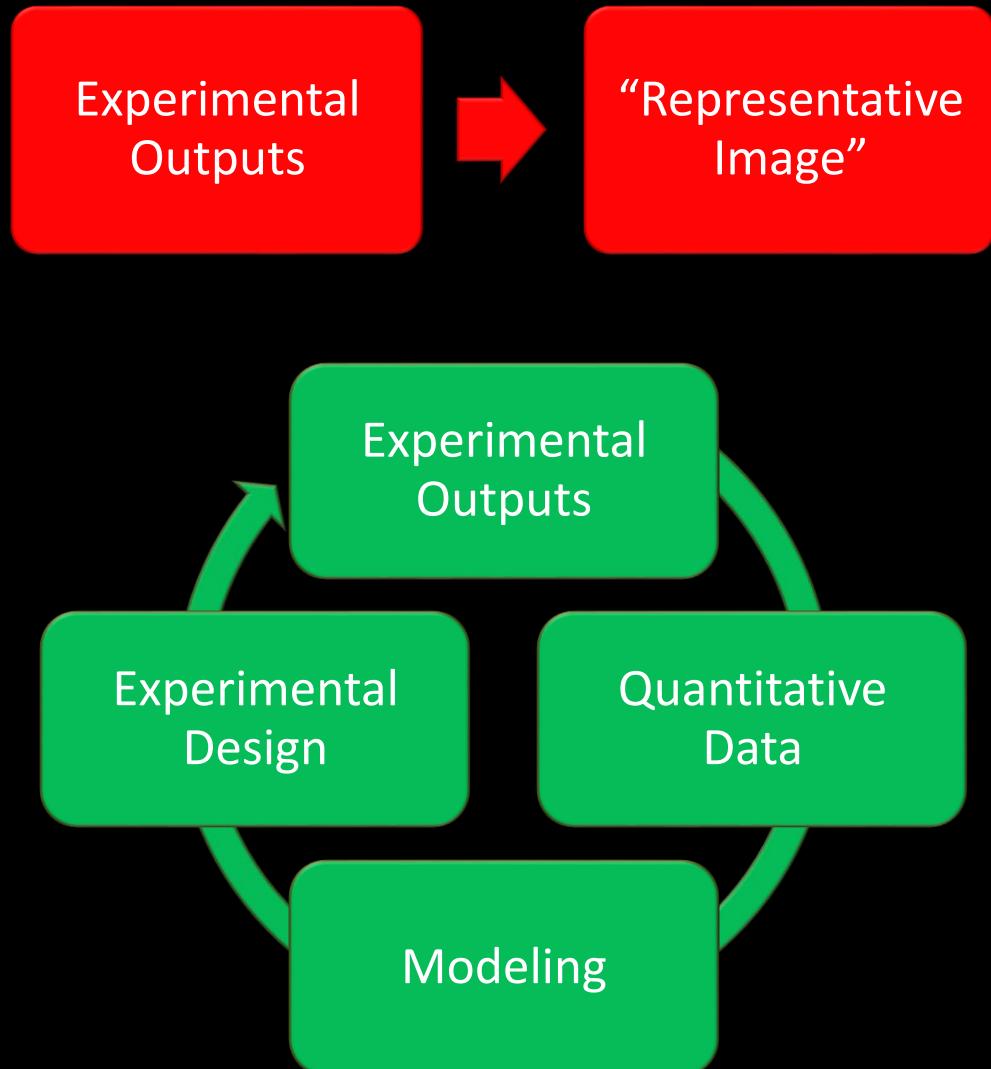
You don't need to take notes – I will send you the slides after the workshop

Please sign the sign-in sheet

Please feel free to ask questions

WHY USE QUANTITATIVE IMAGE ANALYSIS?

- Microscopy historically qualitative
- New technology facilitates quantification
- Limited uptake among biologists



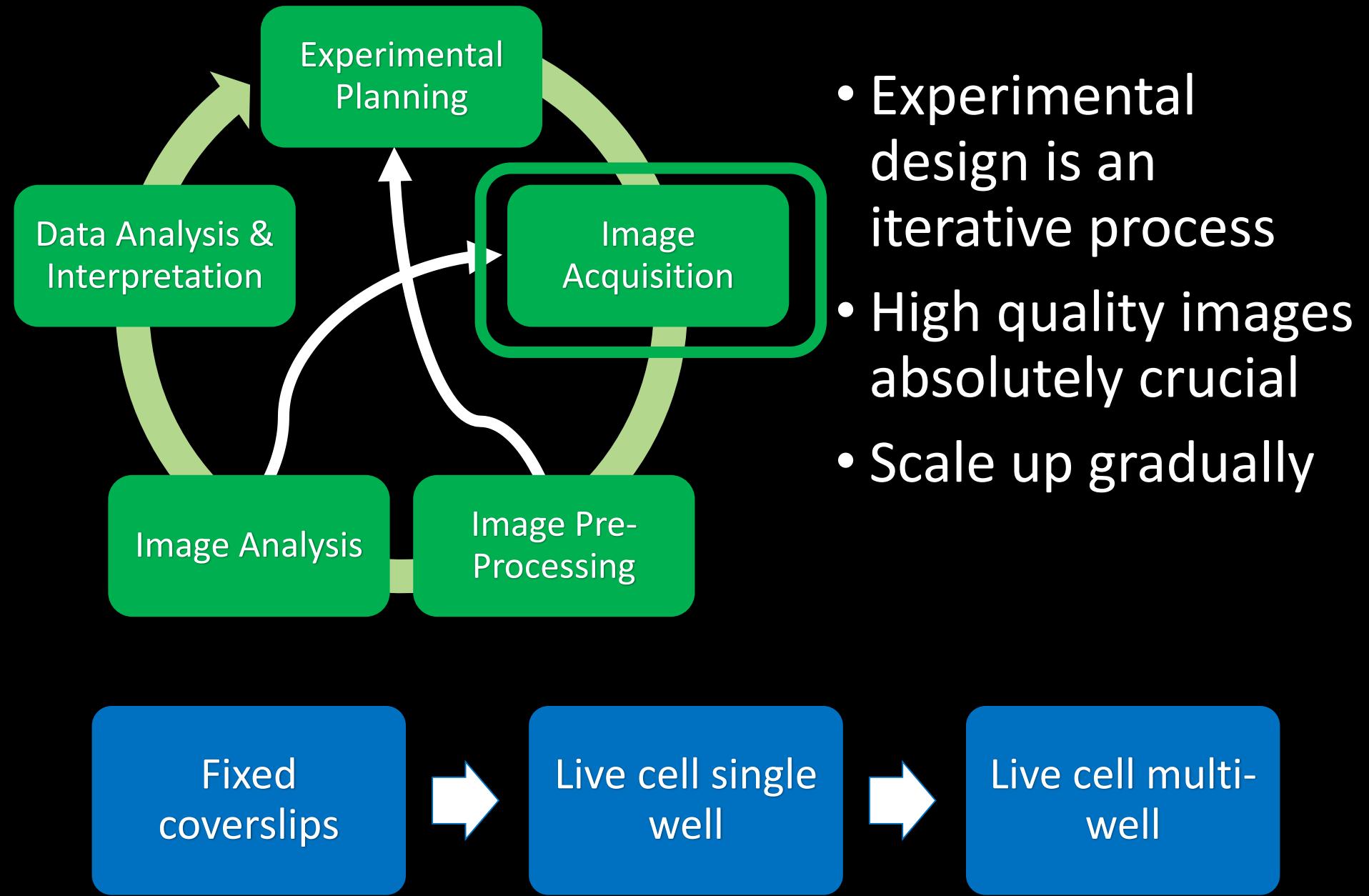
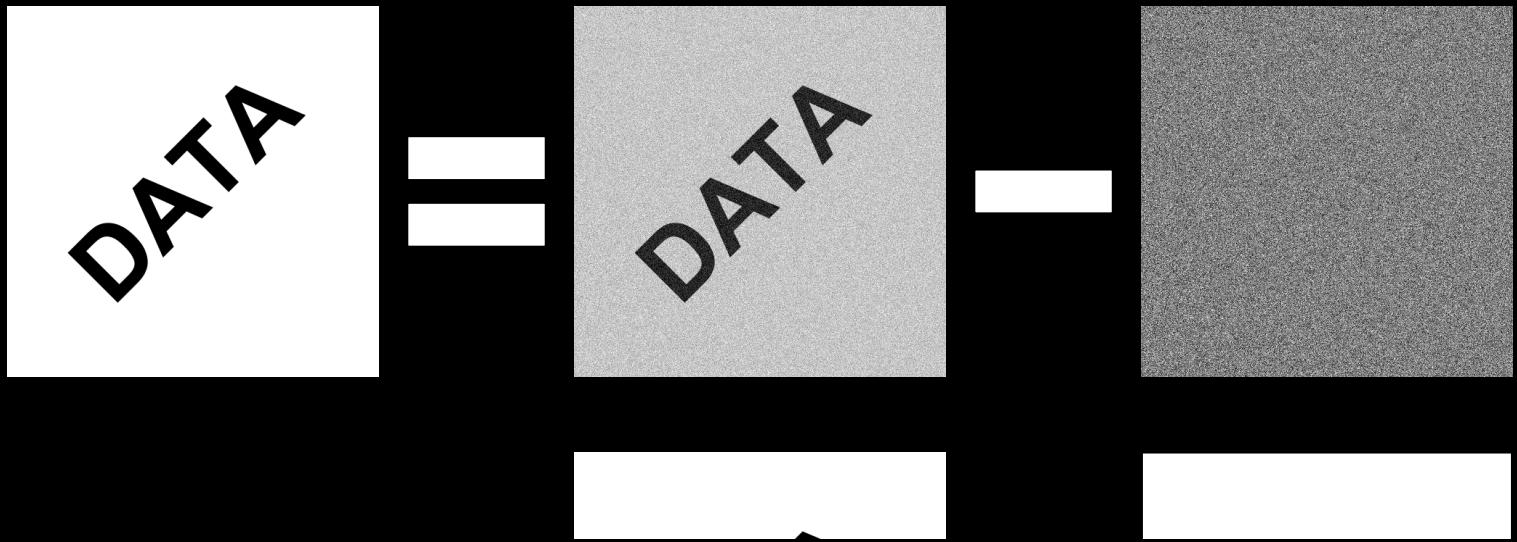


IMAGE ANALYSIS V IMAGE PROCESSING ?

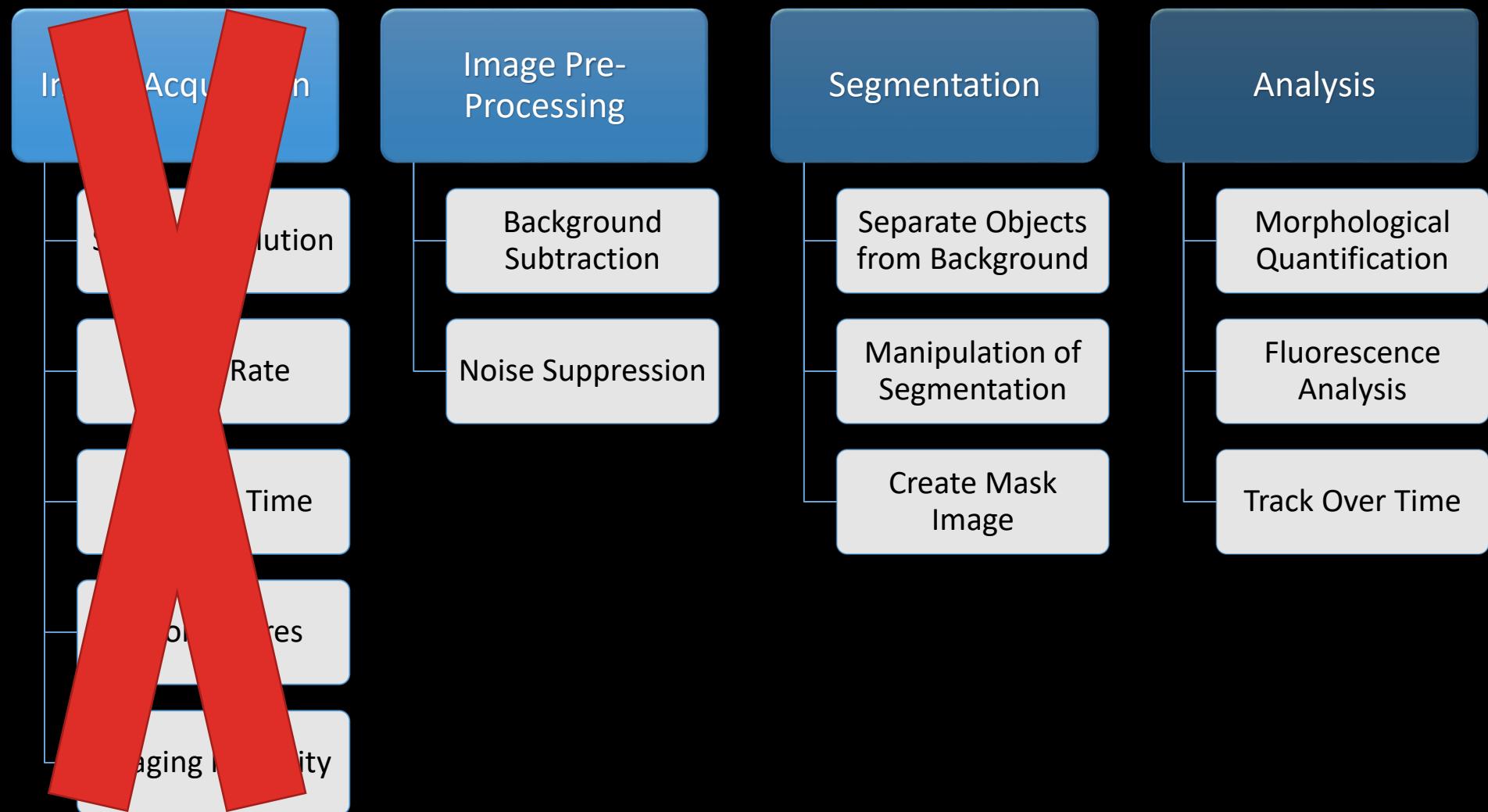
- Image Processing:
 - Enhancement, filtering for noise reduction, image registration, etc.
- Image Analysis:
 - Analysis of data contained within an image. For example, quantification of cell area.

Processing:

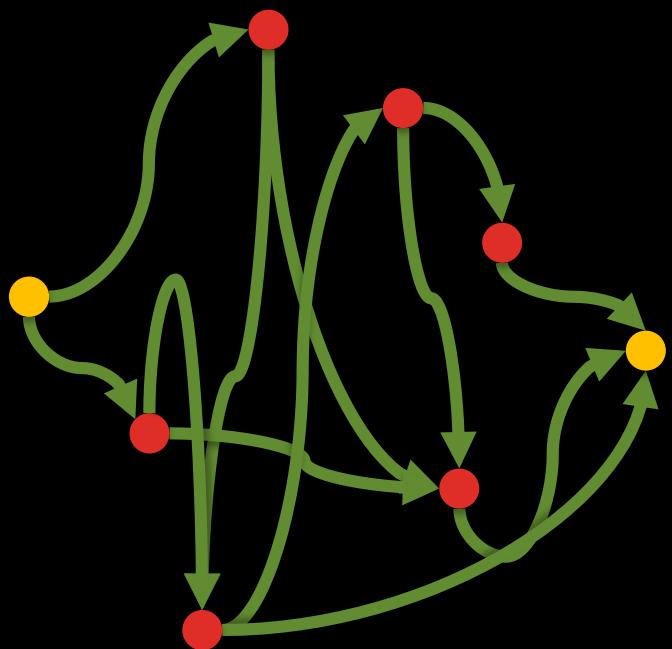


Processing should be kept to a minimum – reduces information content in image

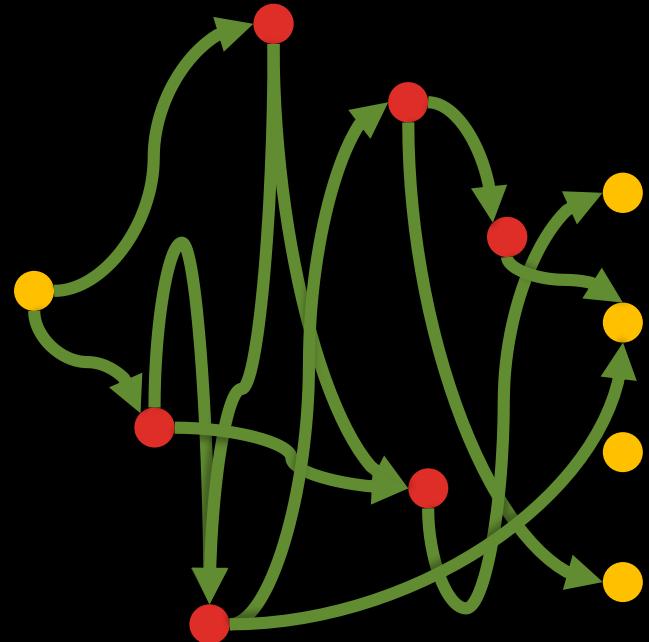
TYPICAL IMAGE ANALYSIS WORKFLOW

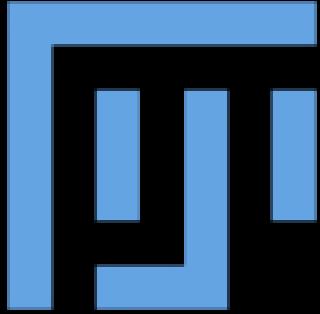


1. There are several different ways to arrive at a solution



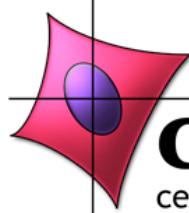
2. There is rarely one single correct solution





- FIJI:
- Fiji Is Just ImageJ
- Actively maintained, with frequent updates.

- ImageJ:**
- Open source
 - Public domain, cross-platform.
 - Read most image formats (via bioformats).
 - Same standard functions as proprietary packages.
 - Open architecture.



CellProfiler
cell image analysis software

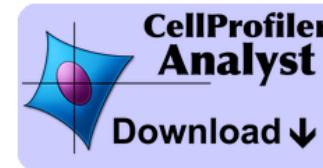


[home](#) [getting started](#) [download](#) [help](#) [about us](#)

[search](#)



CellProfiler is free open-source software designed to enable biologists without training in computer vision or programming to quantitatively measure phenotypes from thousands of images automatically. See our papers on analyzing [cell images](#) and [non-cell images](#).

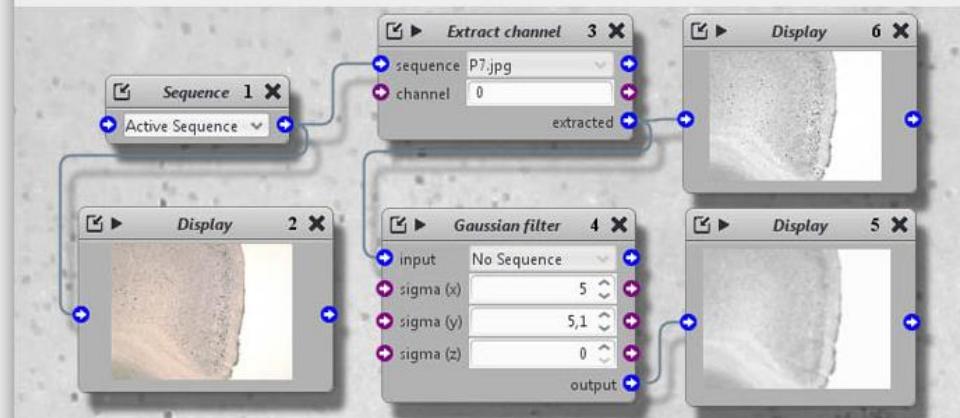


CellProfiler Analyst is free open-source software for exploring and analyzing large, high-dimensional image-derived data. It includes machine learning tools for identifying complex and subtle phenotypes. See our papers on [data visualization](#) and [machine learning](#).

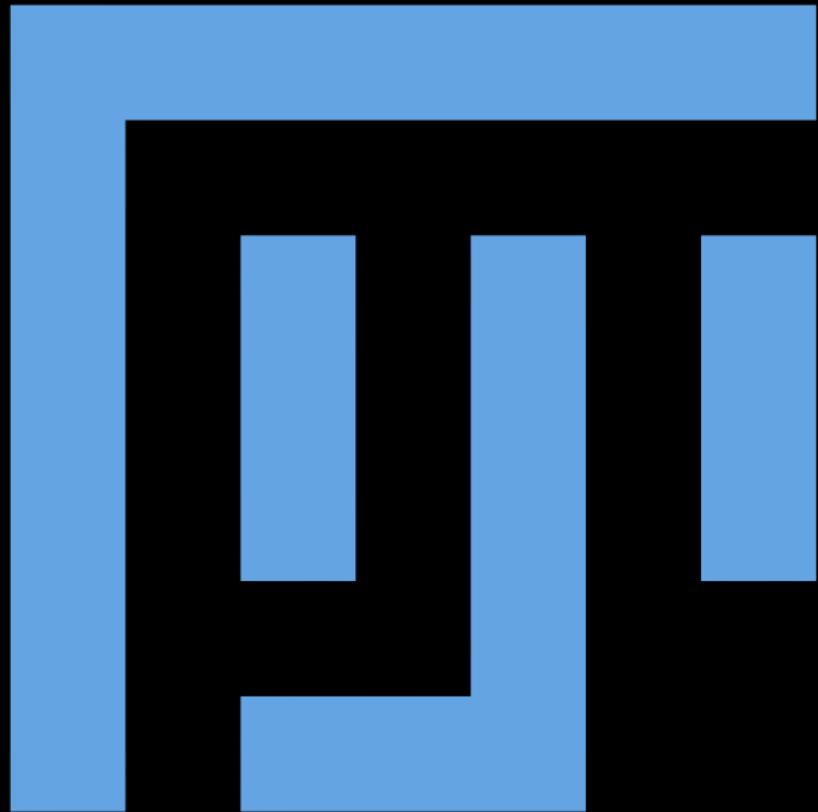
 **icy**

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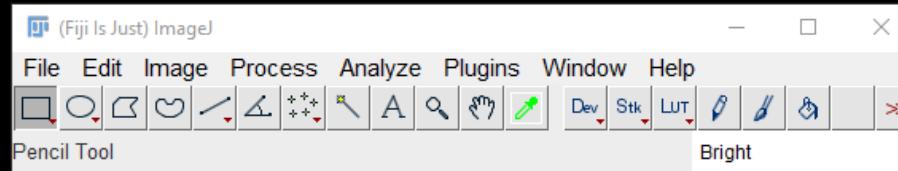


GRAPHICAL PROGRAMMING WITH PROTOCOLS



Let us begin...

IF YOU FORGET HOW TO ACCESS SOMETHING...



Quick Search

Commands
 Brightness/Contrast... /Image/Adjust/Brightness/Contrast...

ImageJ Wiki (8/19)
 Multi Otsu Threshold
 Sholl Analysis
 SoC 2009 Application
 Principles
 Auto Local Threshold
 How to make a new tutorial
 Generate and exploit Kymographs
 RATS: Robust Automatic Threshold Selection

ImageJ Forum

Brightness/Contrast...

Menu path
Image > Adjust > Brightness/Contrast...

Shortcut
control shift C

Identifier
legacy:ij.plugin.frame.ContrastAdjuster

Location
/C:/Users/barryd/FIJI/FIJI-N~1/Fiji.app/jars/ij-1.52a.jar

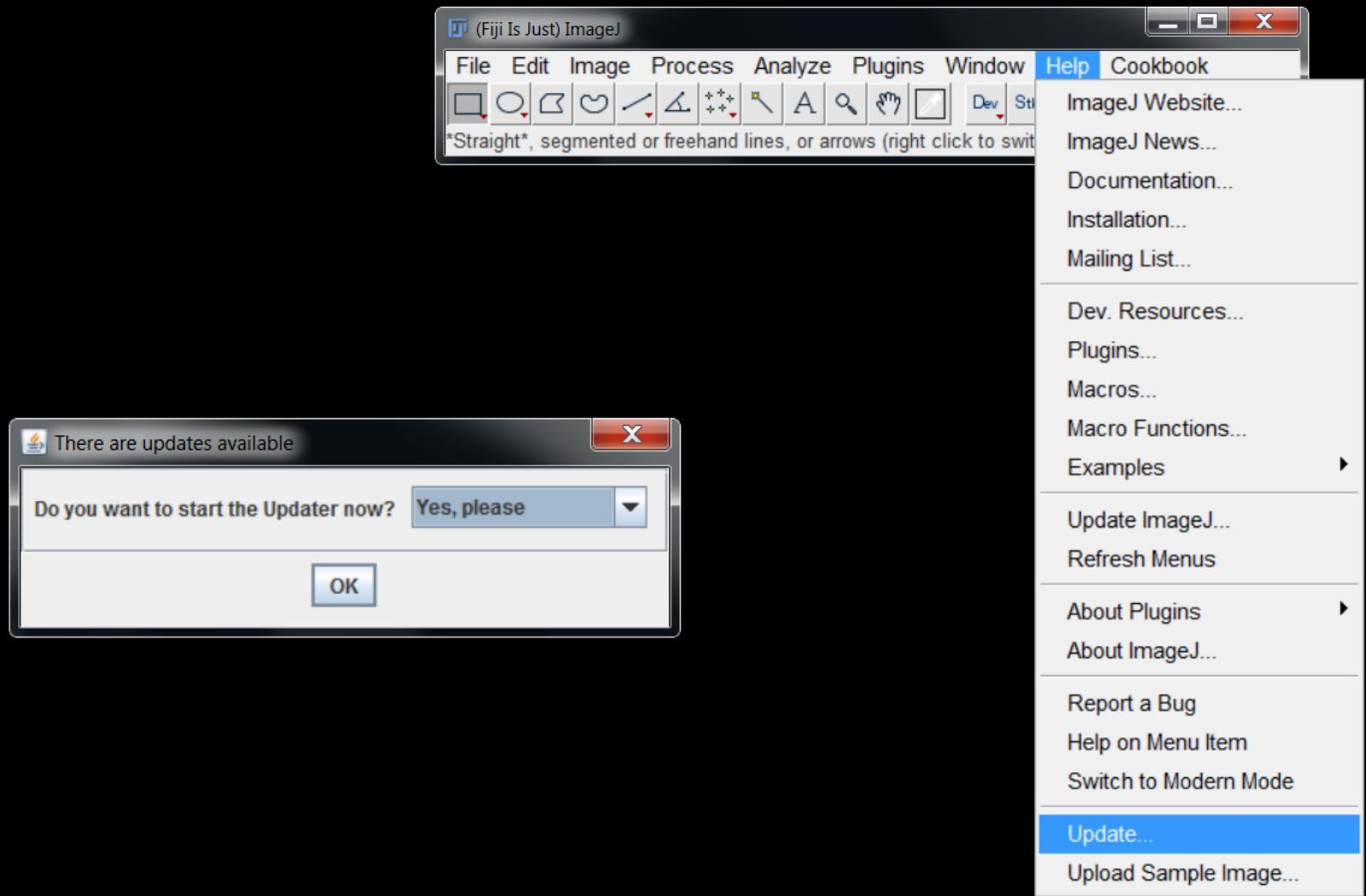
Run

Help

Source

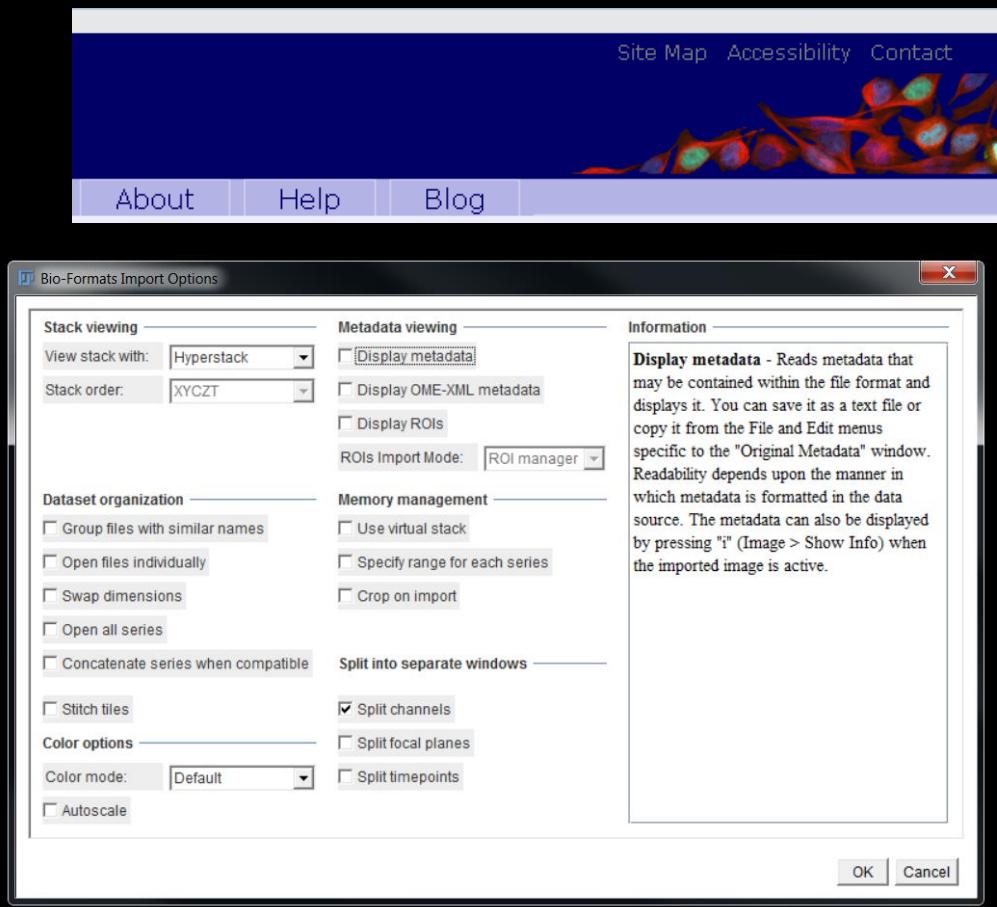
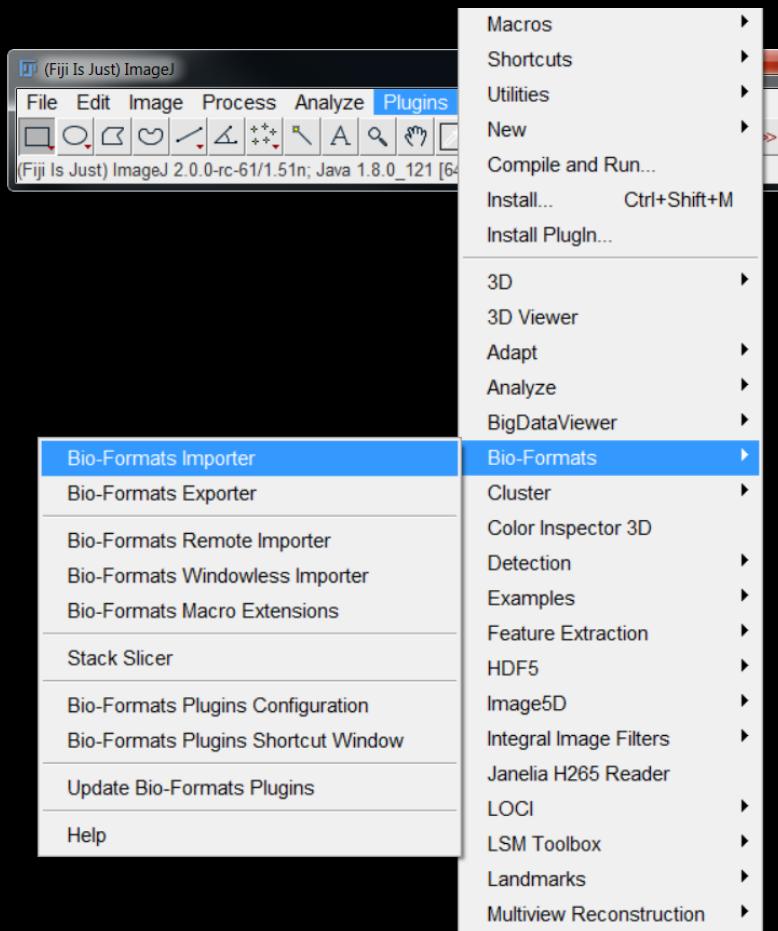
A detailed screenshot of the "Quick Search" dialog in Fiji. On the left is a list of search results under categories like Commands, ImageJ Wiki, and ImageJ Forum. The first result, "Brightness/Contrast...", is highlighted with a blue background and has its details displayed on the right. The right panel shows the "Identifier" as "legacy:ij.plugin.frame.ContrastAdjuster" and the "Location" as the path to the Fiji jar file. At the bottom of the dialog are buttons for "Run", "Help", and "Source".

UPDATING FIJI



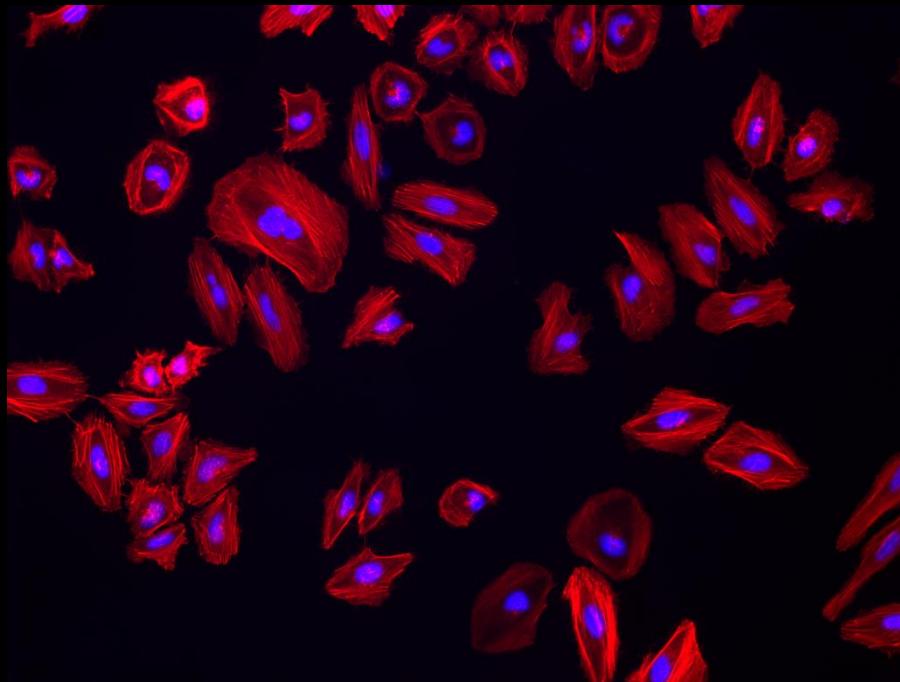
OPENING AN IMAGE

- Don't Drag & Drop
- Bioformats ensures consistent reading of metadata
 - Independent project distributed with FIJI

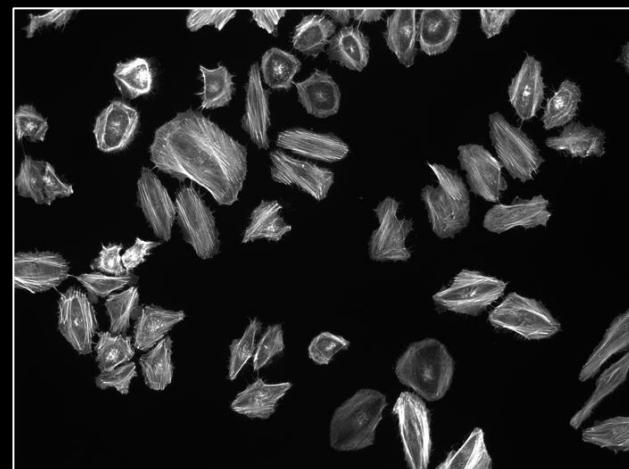
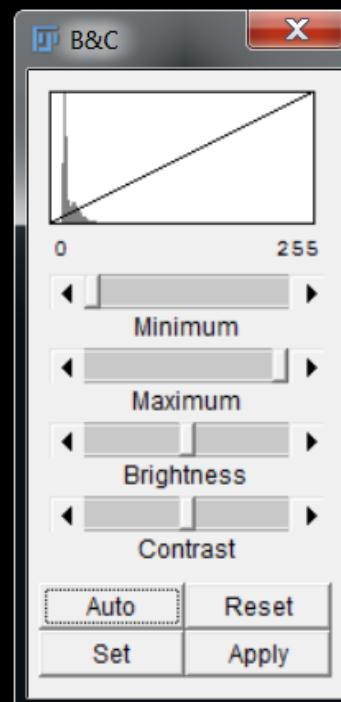
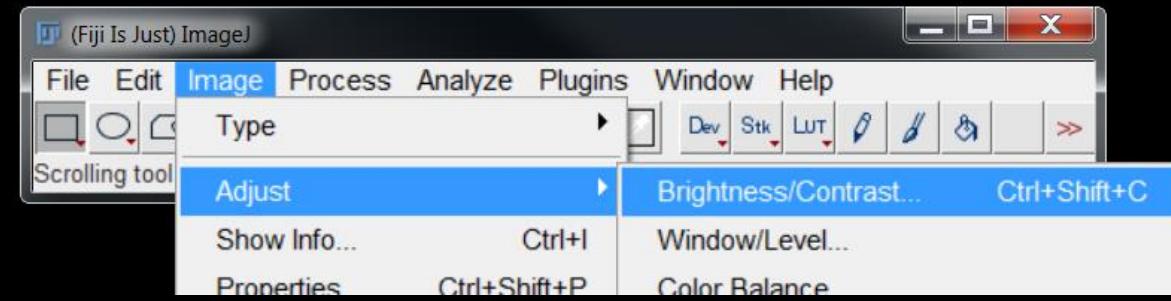


DEMO 1 – OPENING IMAGES WITH BIOFORMATS

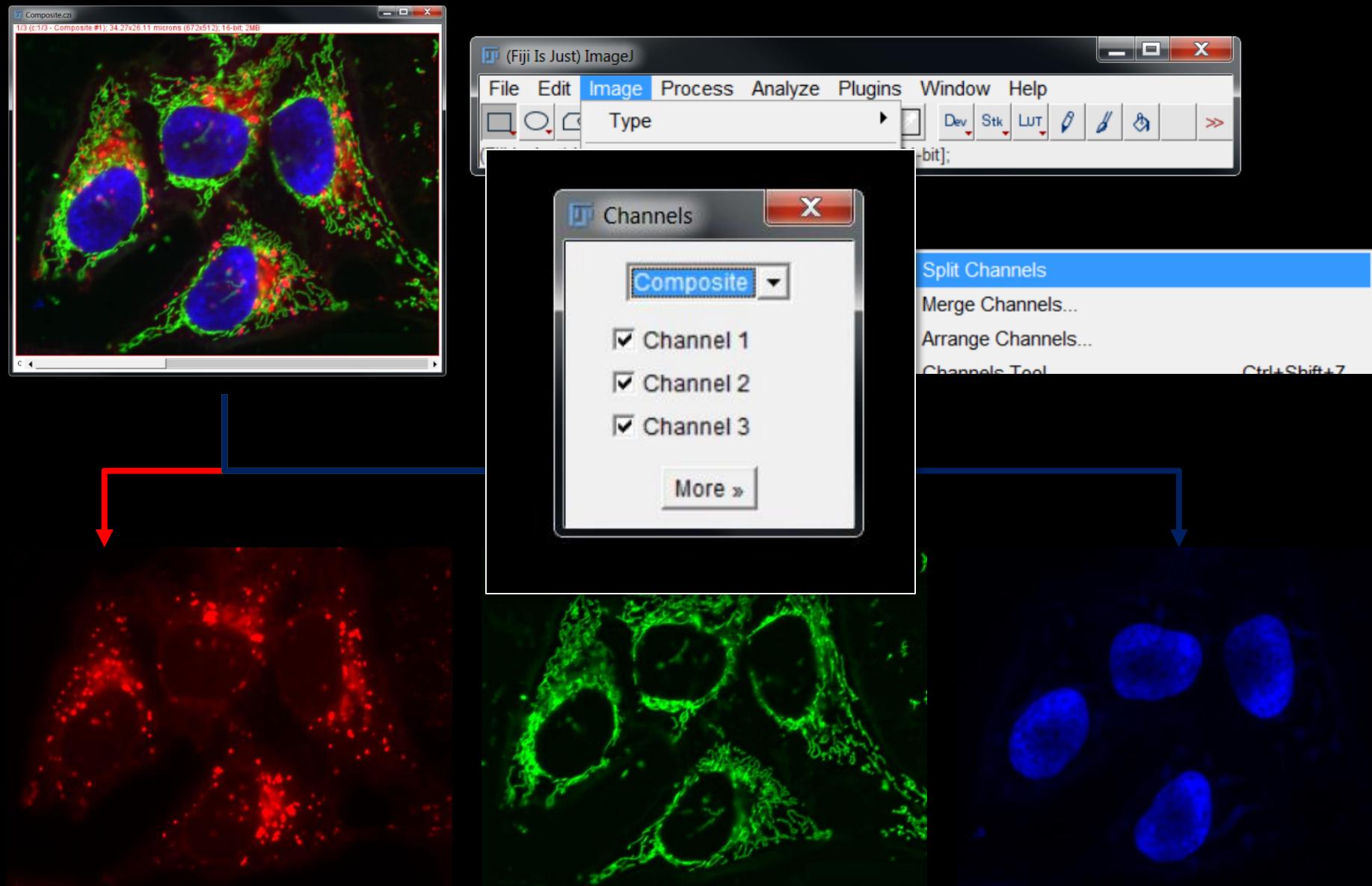
- Import Metamorph (TIFF) Dataset:
 - Overview of various options presented in Bioformats GUI
 - Illustration of reading of MetaData
 - Images can *appear* black, but pixels have values greater than zero



ADJUSTING CONTRAST



WORKING WITH CHANNELS



LOOK-UP TABLES (LUTS)

By default, pixel values are mapped to grey levels...

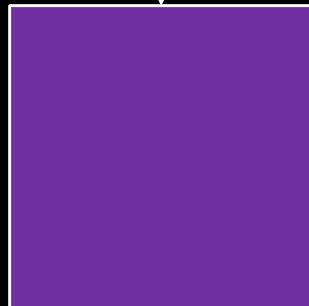
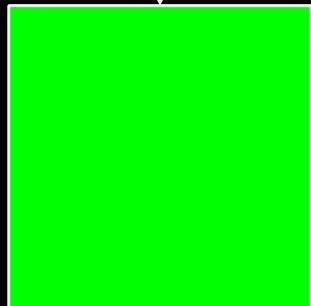
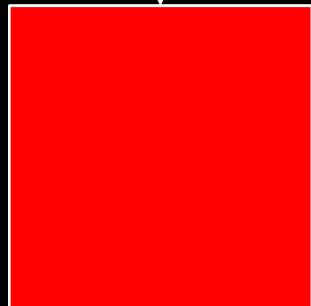
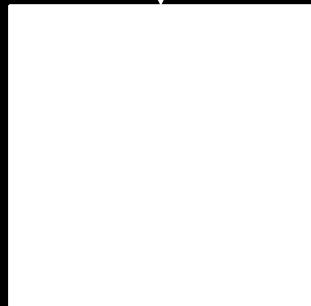
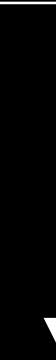
0

64

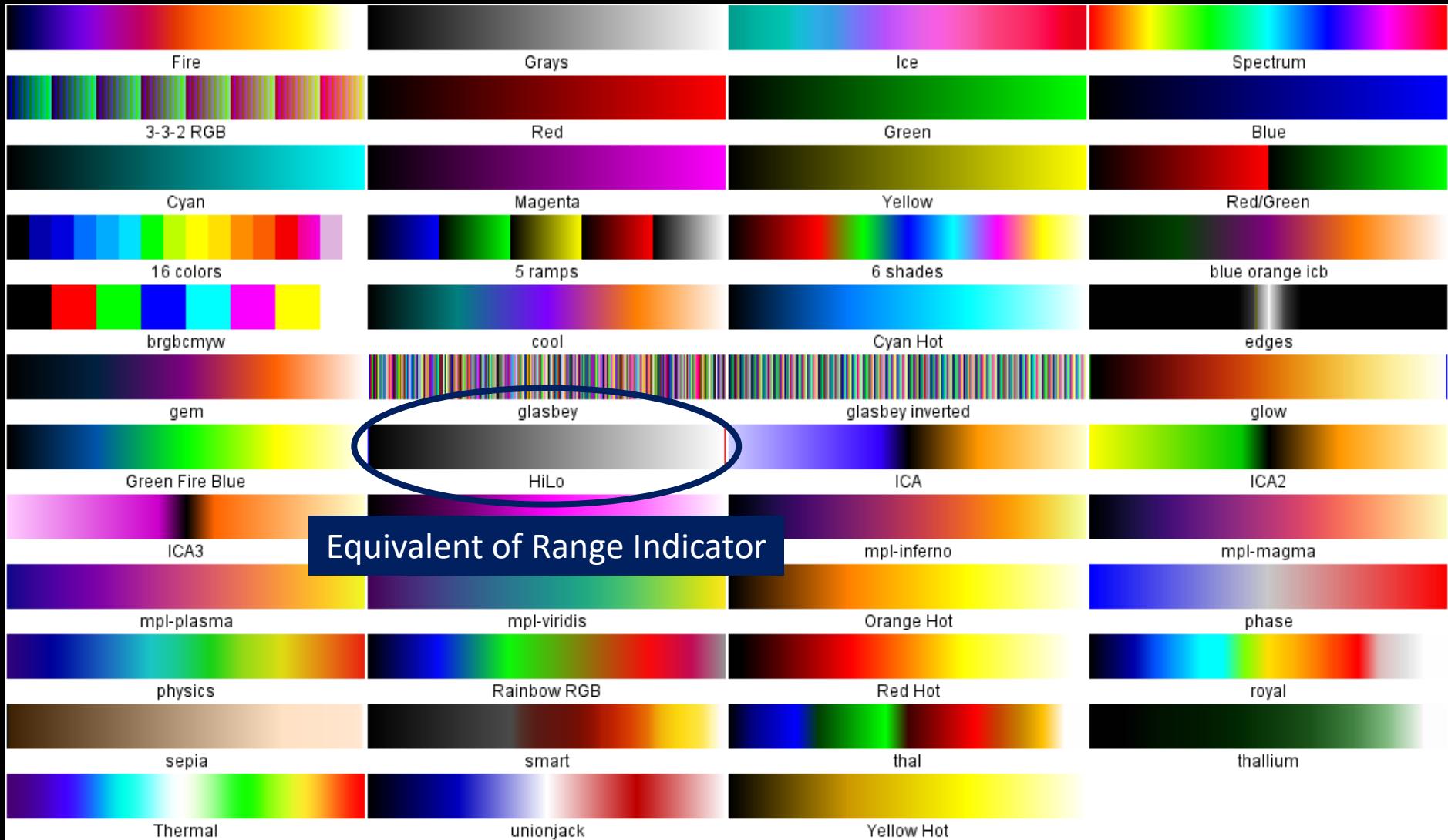
128

192

255



...but there is no reason why this has to be the case.

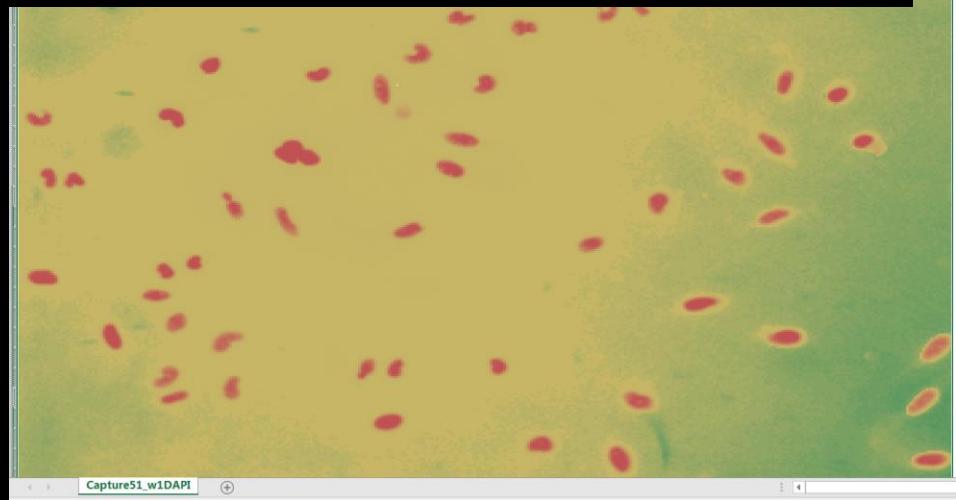
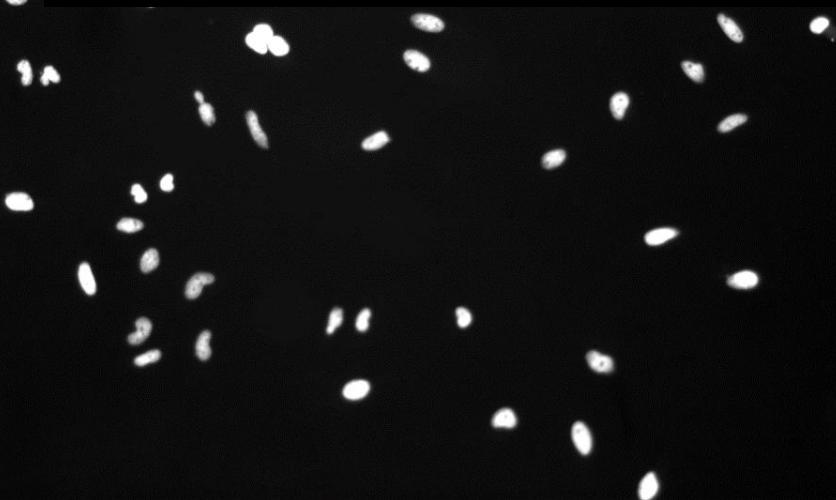


IMAGES ARE JUST NUMBERS

“Solutions to image analysis problems often appear simple, particularly when easily accomplished by the human visual system, which is complex and poorly understood.”

The human brain is excellent at pattern matching...

...but this can result in us seeing “patterns” that don’t really exist

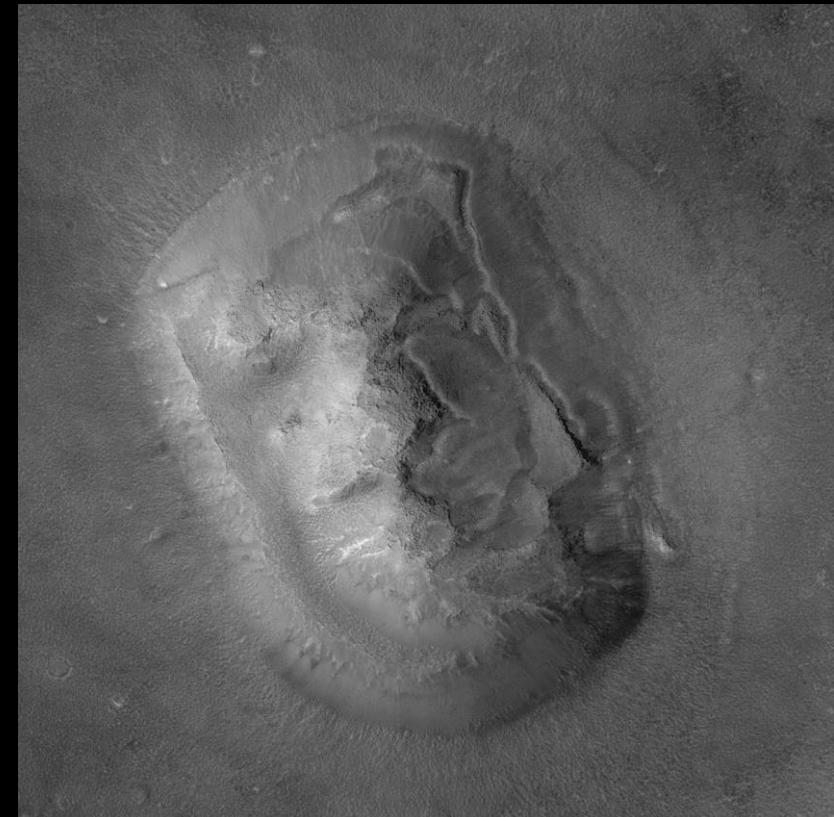


THIS CAN CAUSE PROBLEMS...

Pareidolia is a psychological phenomenon in which the mind responds to a stimulus by perceiving a familiar pattern where none exists (e.g., in random data).



1976, *Viking 1*

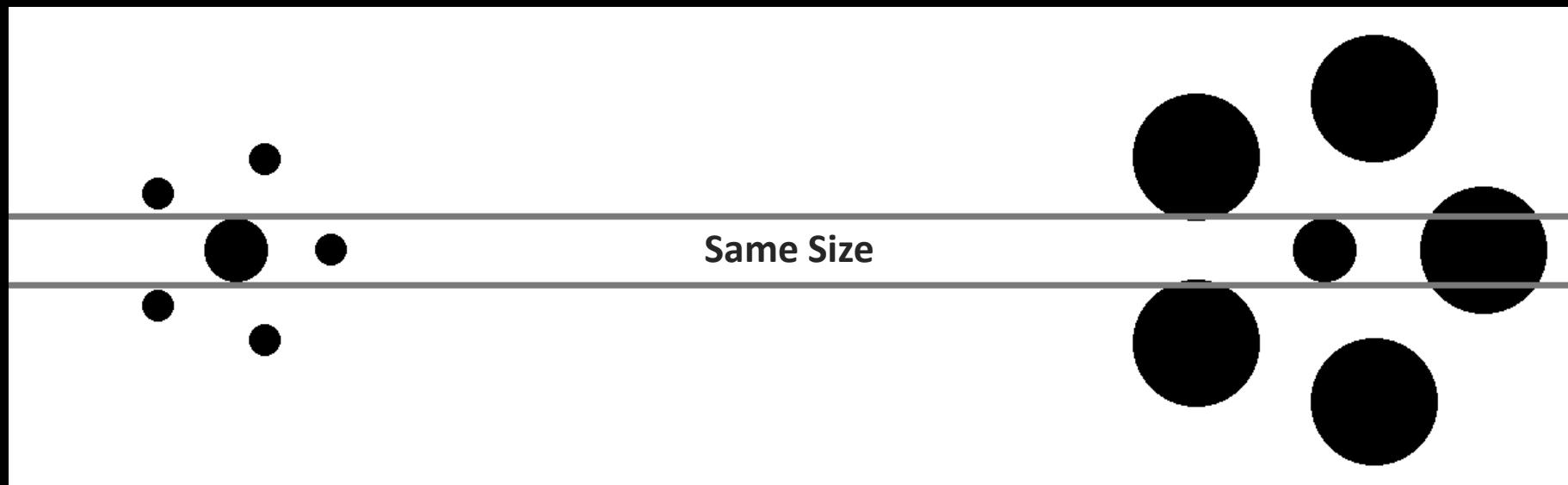


2001, *Mars Global Surveyor*

Which nucleus is brighter?



Which centre circle is bigger?



WHAT'S YOUR POINT DAVE?!?!

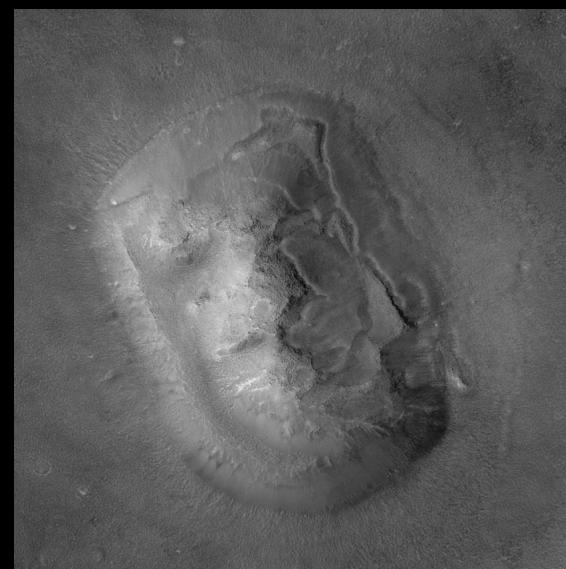
We're here to learn image analysis – you're preaching to the choir!

People will very often design an analysis pipeline to confirm what **they think they see** visually....

...which often results in significant frustration.



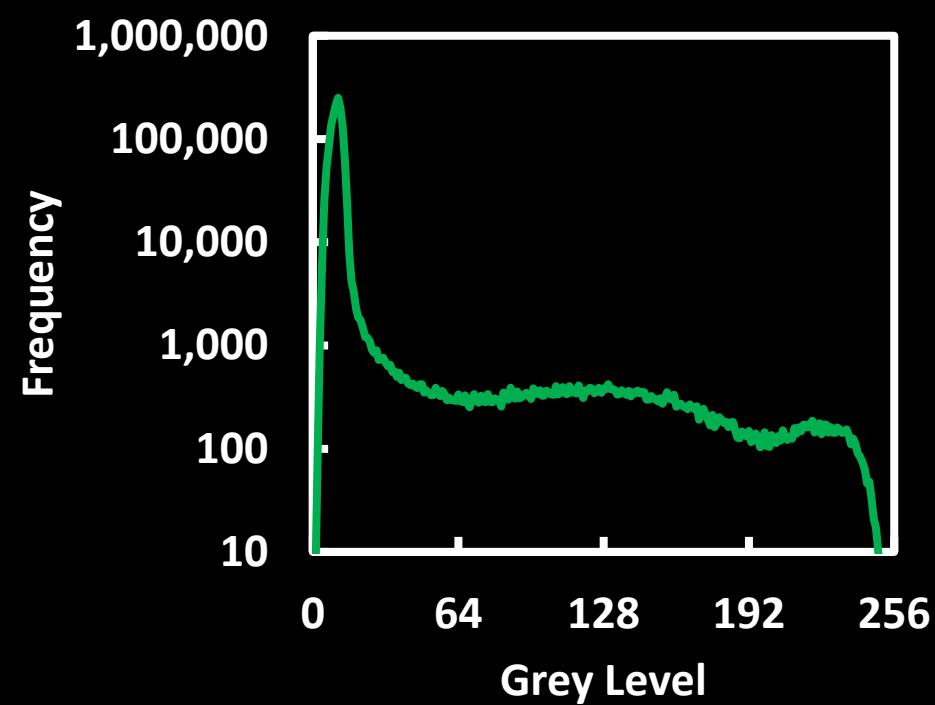
Expected Result: Humanoids on Mars



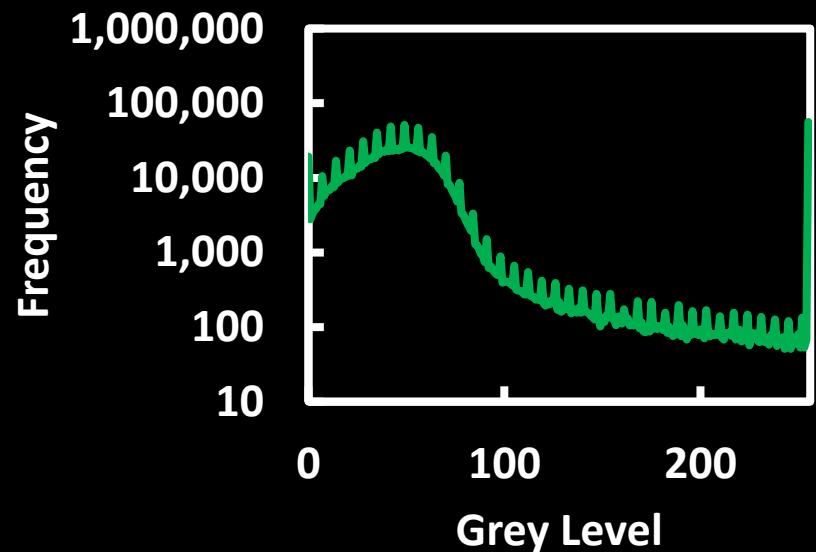
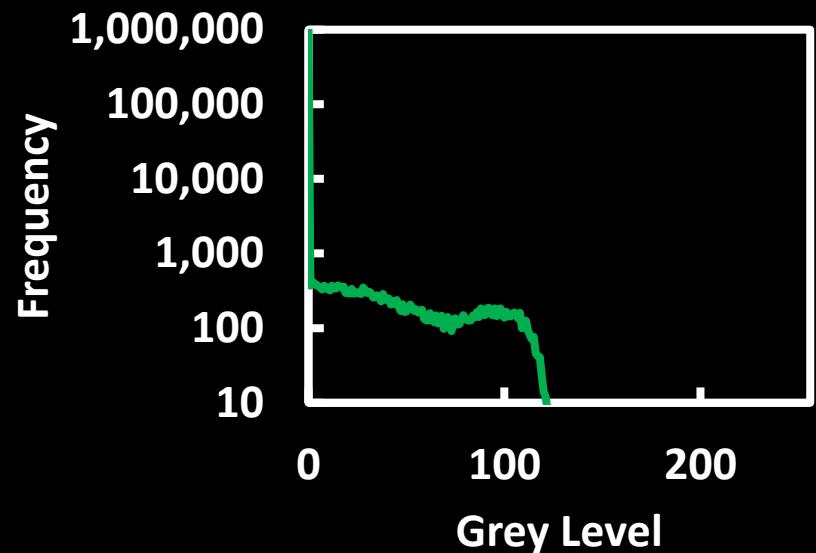
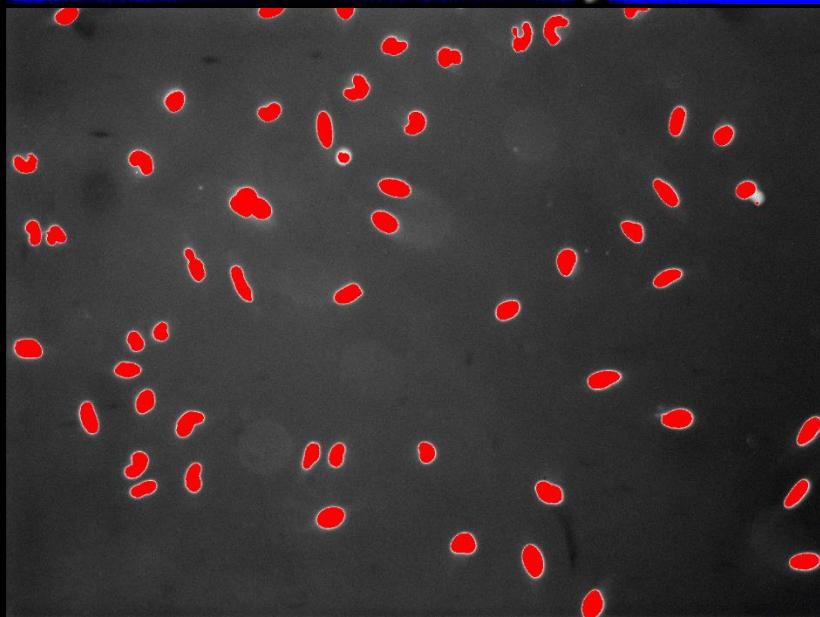
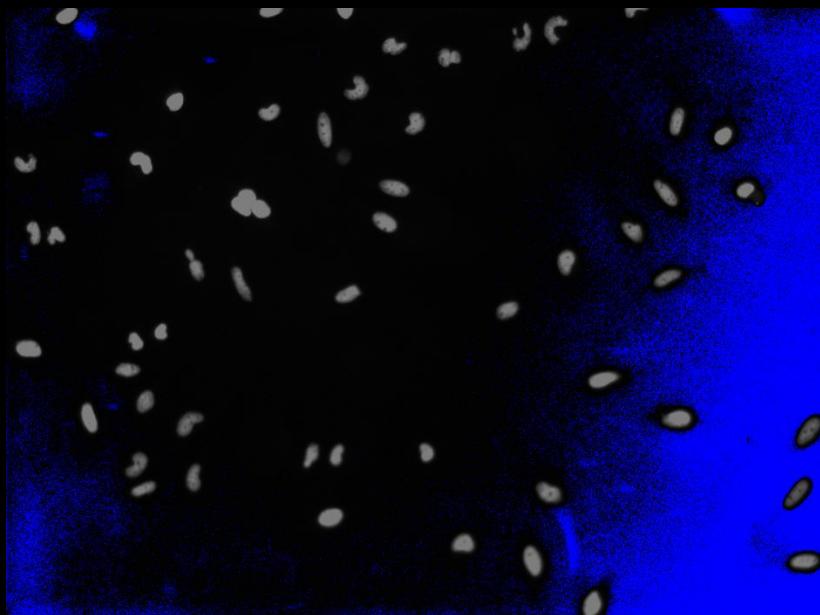
Actual Result: Big Rock

HISTOGRAMS

A graphical representation of the distribution of numerical data



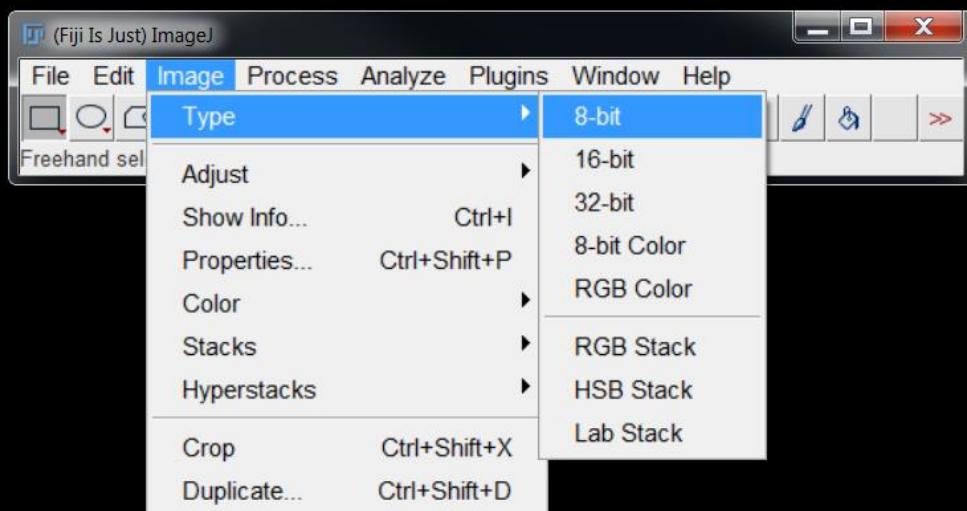
INTERPRETING HISTOGRAMS



BIT DEPTH

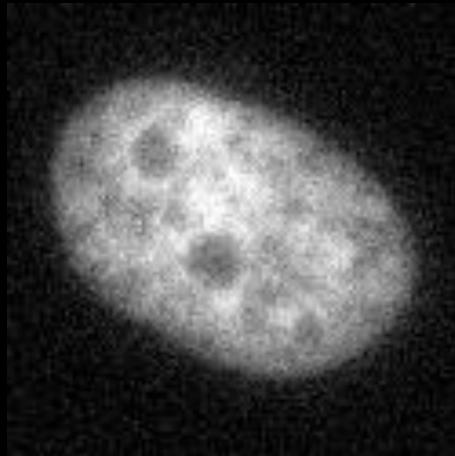
Range of values a pixel can represent

Bits per pixel	Number of values	Range of values
8	2^8	0 – 255
12	2^{12}	0 – 4,095
16	2^{16}	0 – 65,535
32	2^{32}	0 – 4,294,967,295

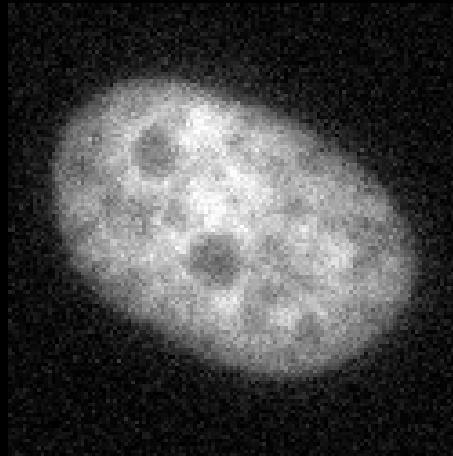


**Greater bit depth
means larger file
sizes**

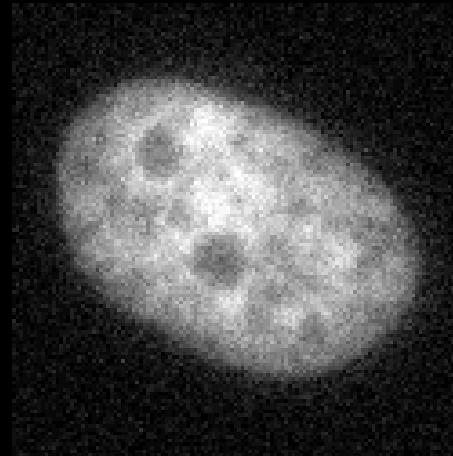
REDUCING BIT DEPTH LOSES INFORMATION



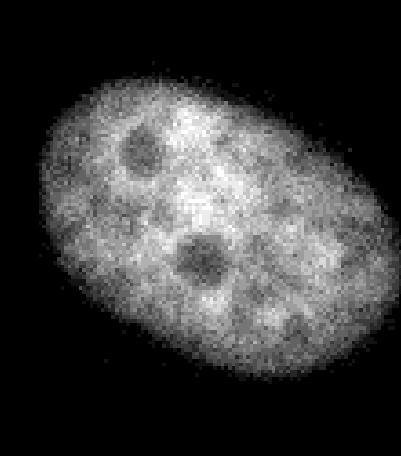
8-bit



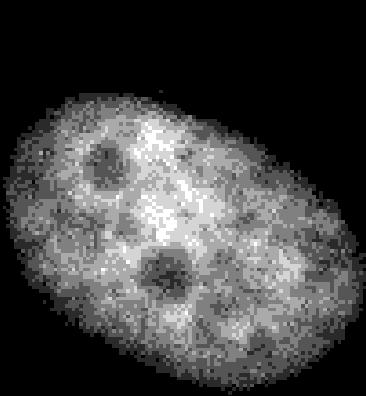
7-bit



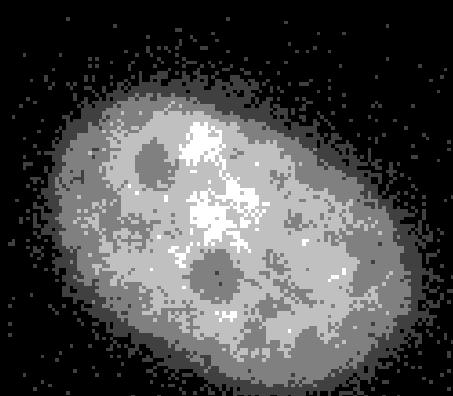
6-bit



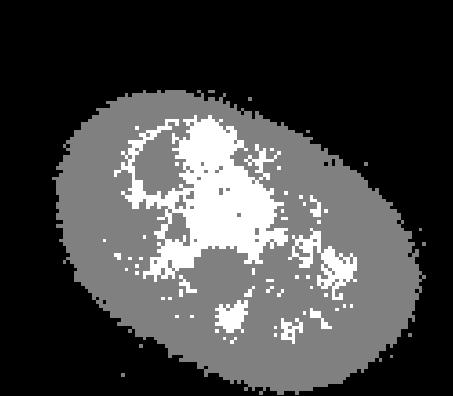
5-bit



4-bit



3-bit



2-bit



1-bit

DEMO 2 – MANIPULATING PIXEL VALUES

- Reducing bit depth incurs loss of data
- Best to deal with raw values
 - Operations performed more precise (e.g. lower rounding error)
- Compress for presentation

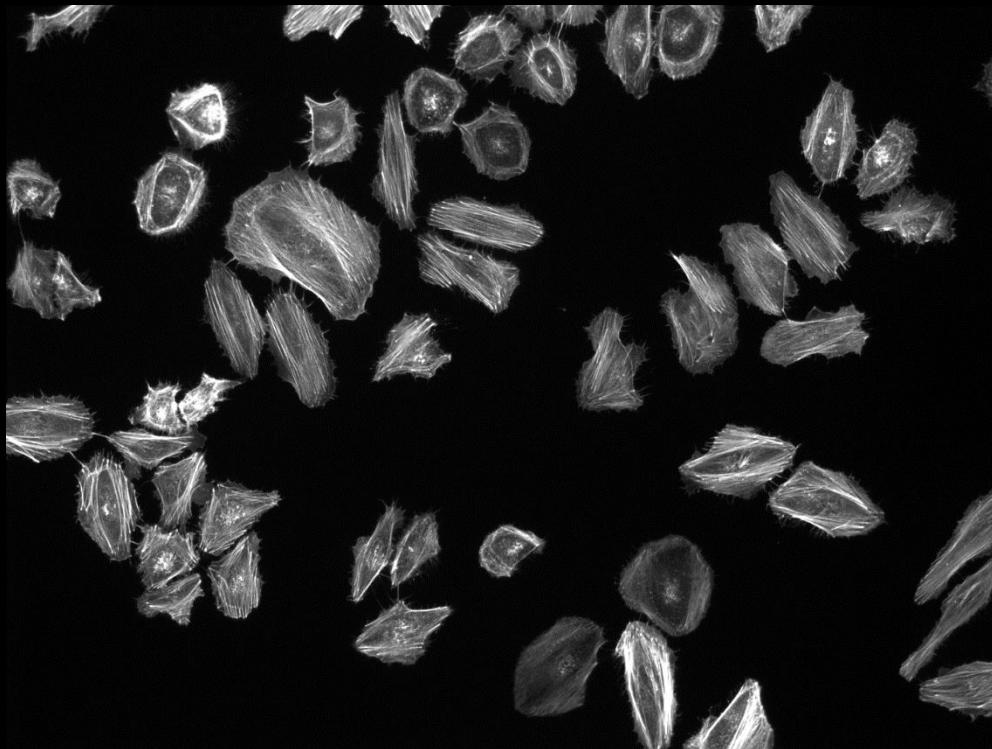


IMAGE COMPRESSION



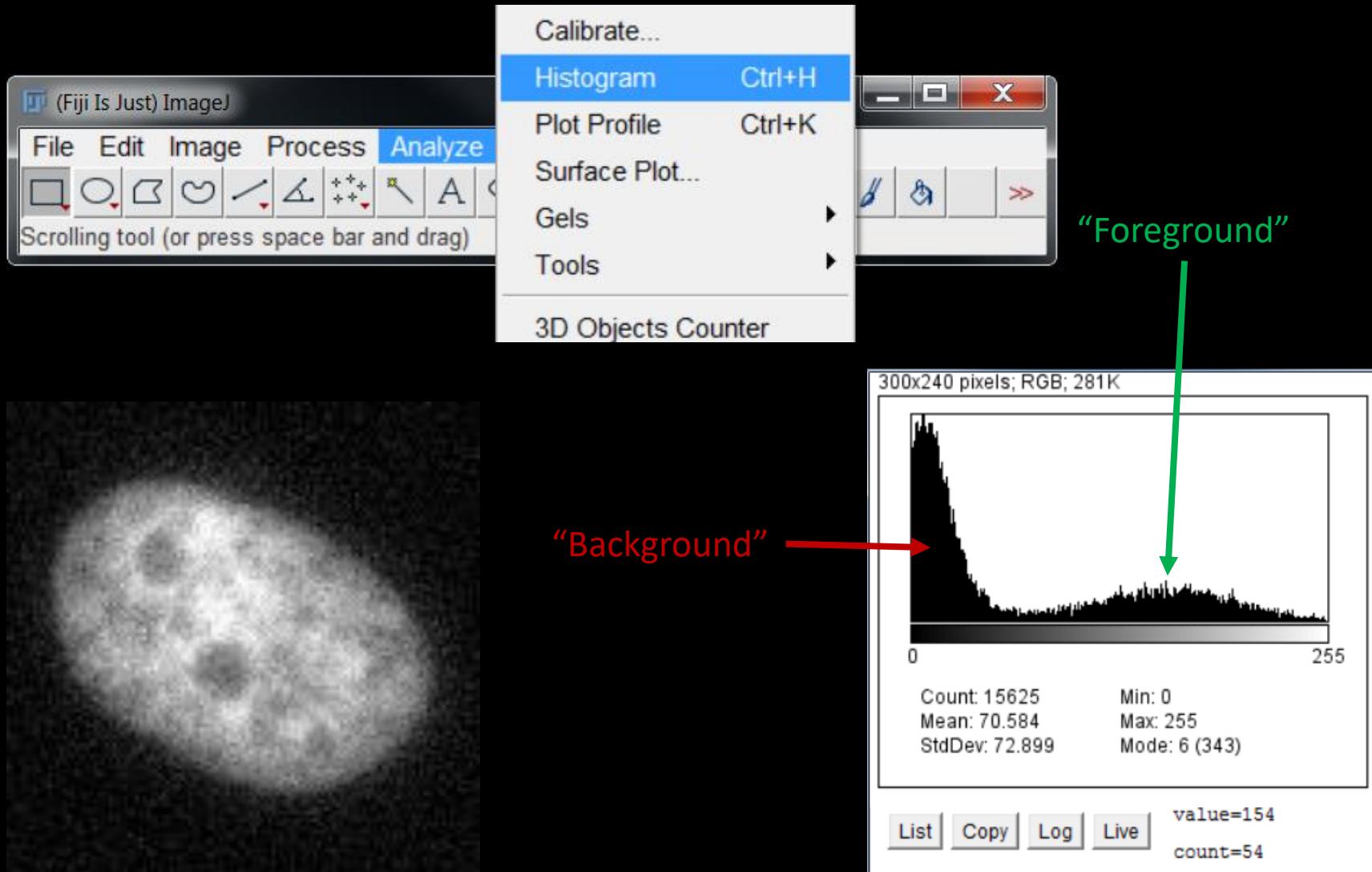
- Lossless:
 - All image information is preserved when saved.

Lossy Compression: Anything that changes pixel values



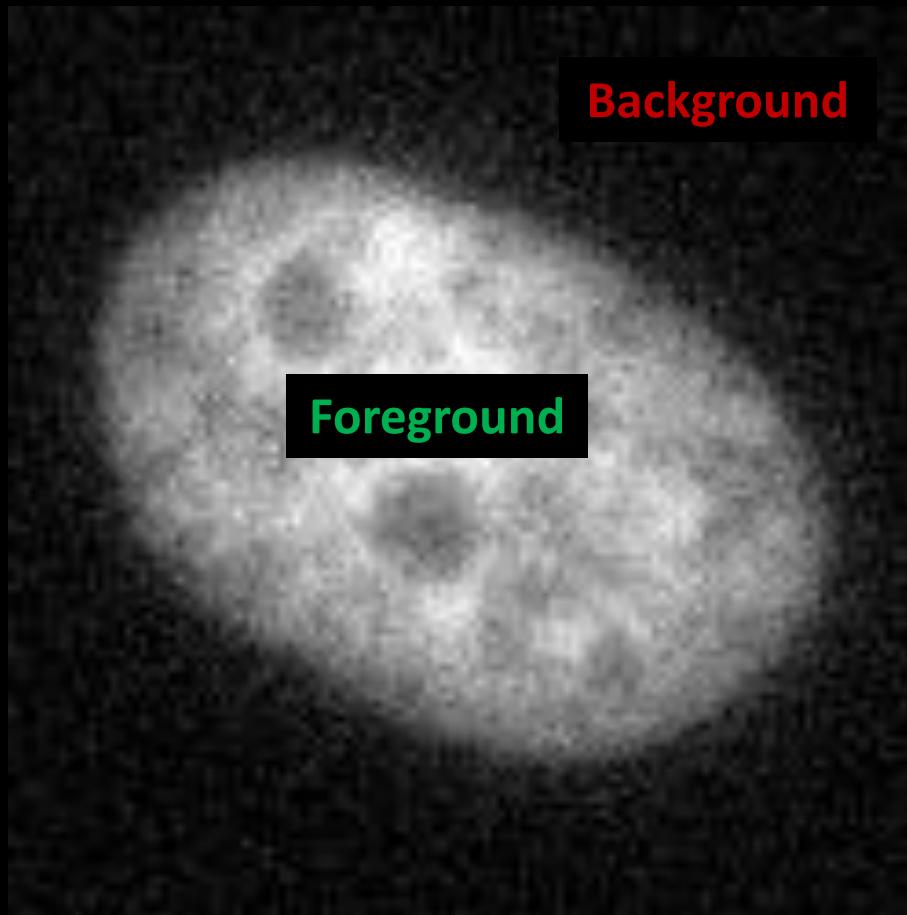
- Image information is irreversibly lost when saved.
- JPEG, GIF
- Typically results in small file sizes

INTERPRETING HISTOGRAMS FURTHER

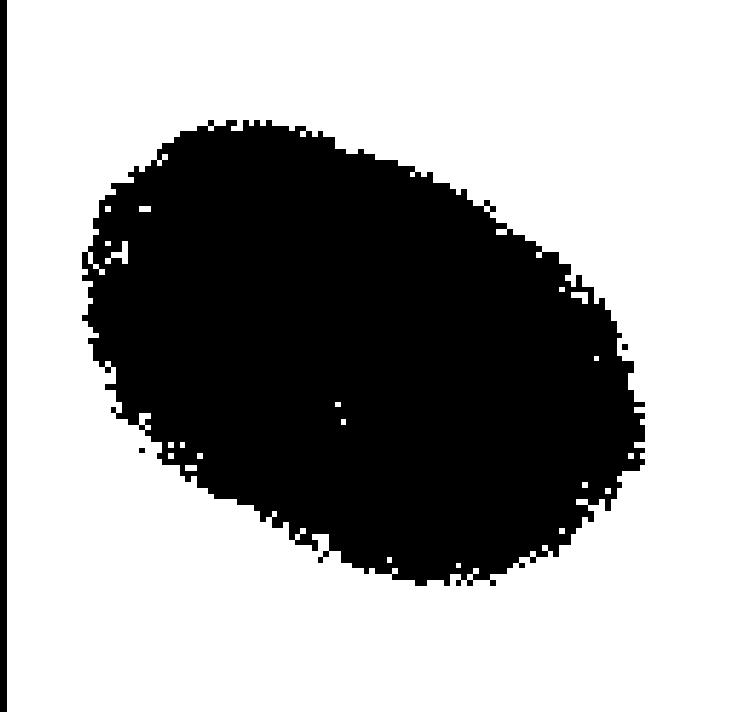
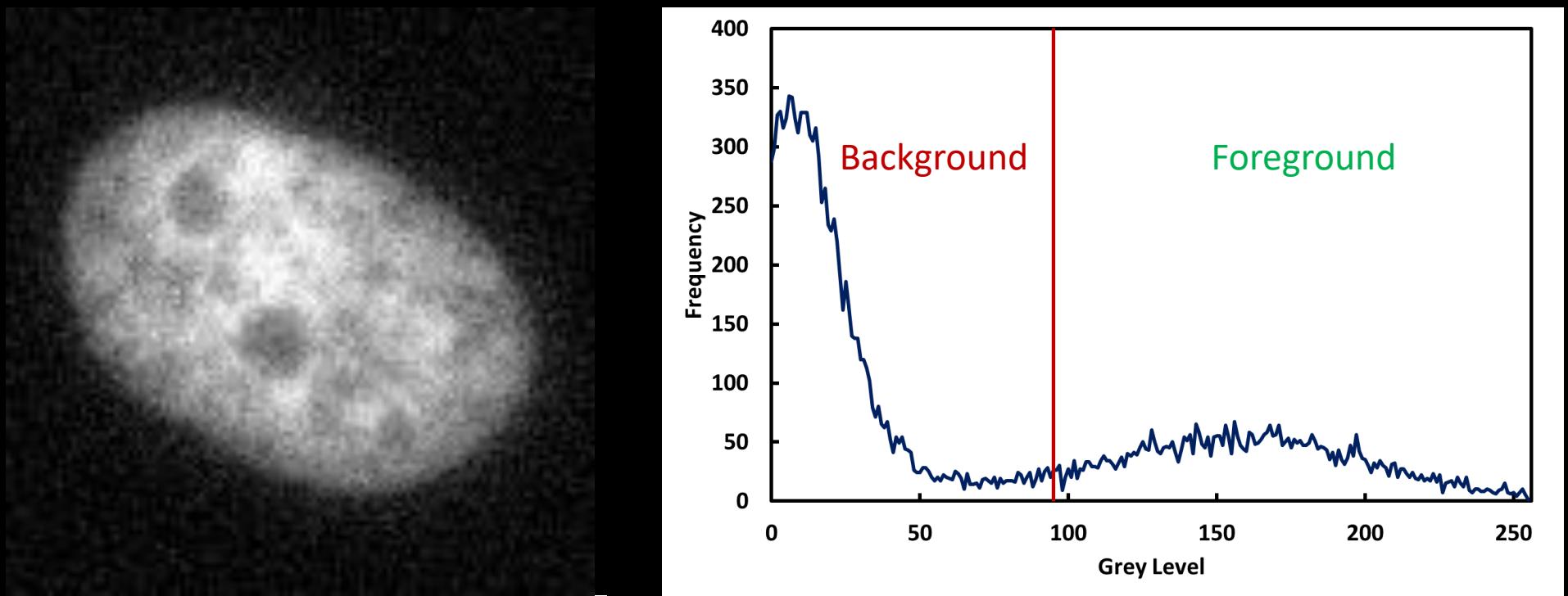


Can be exploited for segmentation

WHAT IS IMAGE SEGMENTATION?

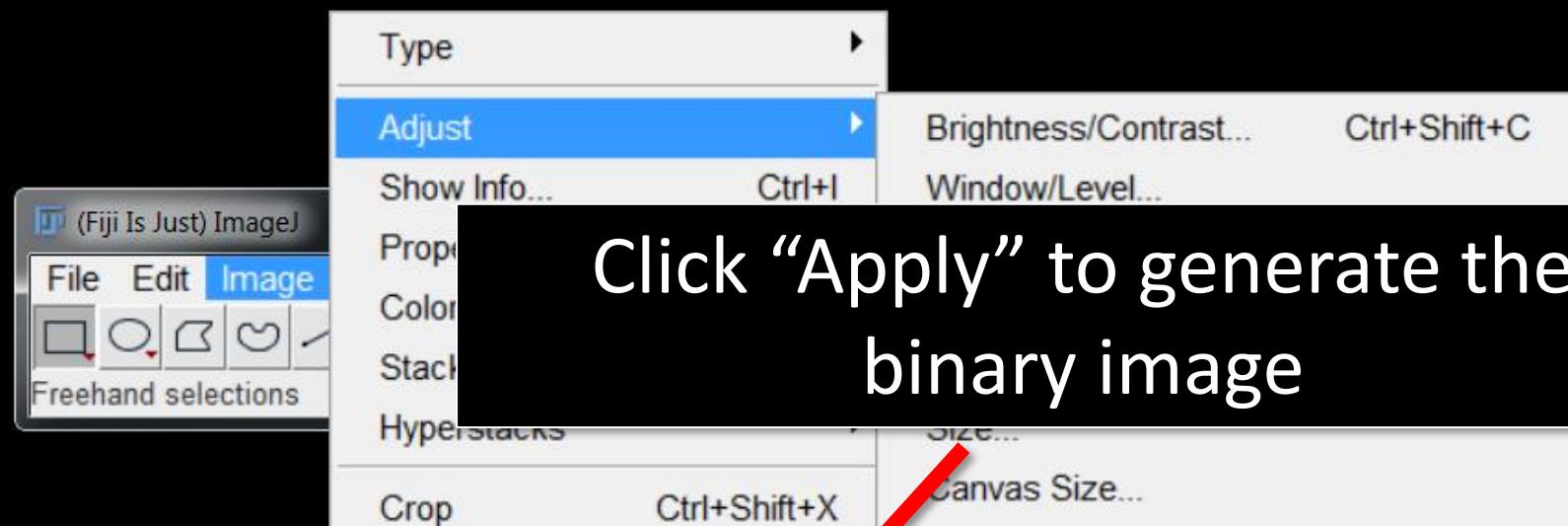


- The process of dividing an image into different regions
- Assigning a label to each pixel within an image
- Pixels within a region should have similar properties

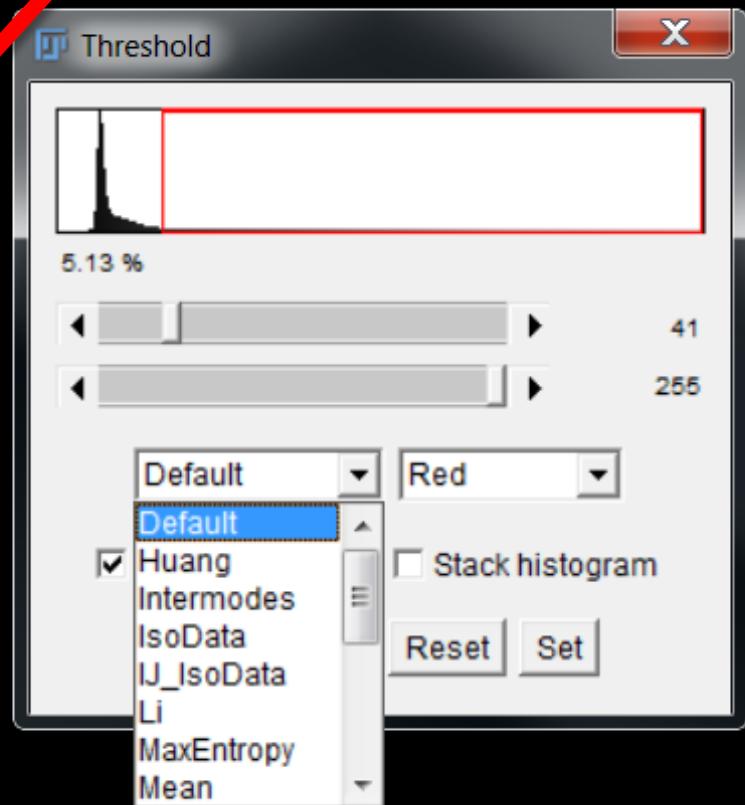
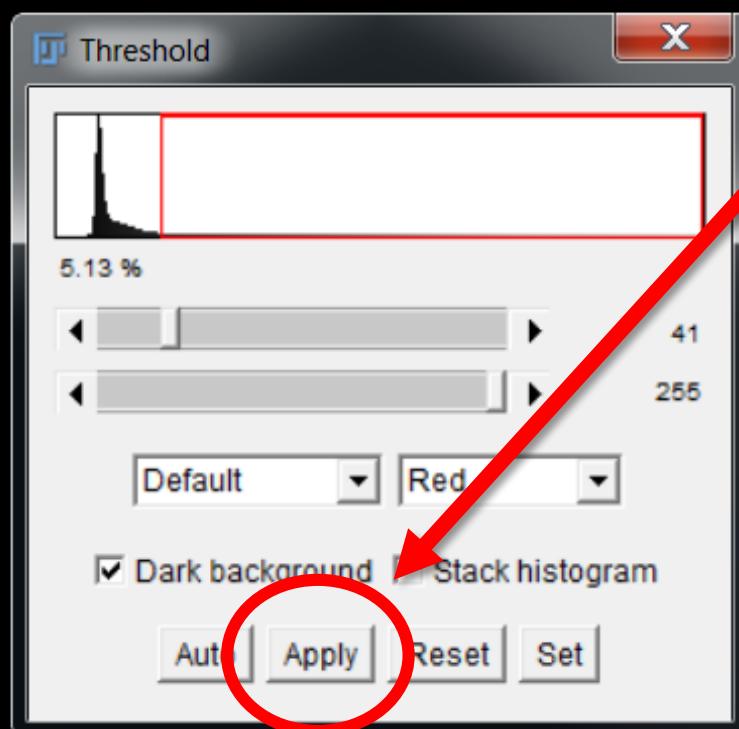


$$T > \frac{\text{Average of Foreground Pixels} + \text{Average of Background Pixels}}{2}$$

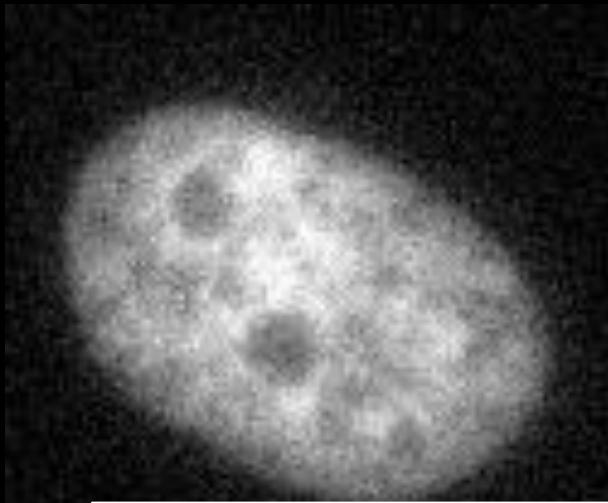
- FIJI has several variations on this algorithm
- Each produces different results
- **Result referred to as binary (or mask) image**



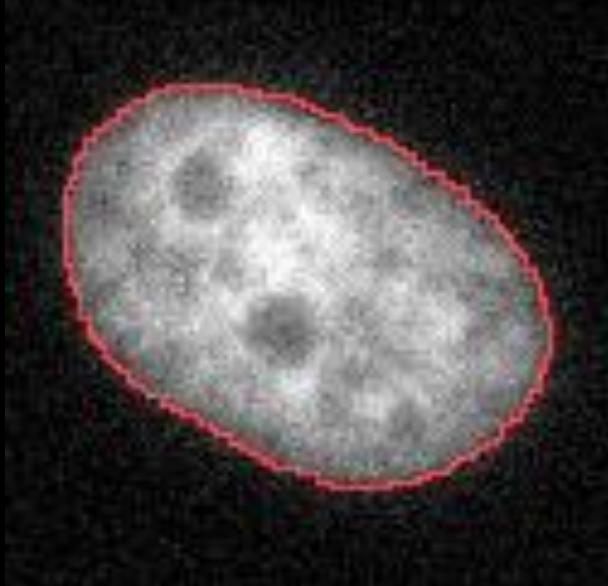
Click “Apply” to generate the binary image



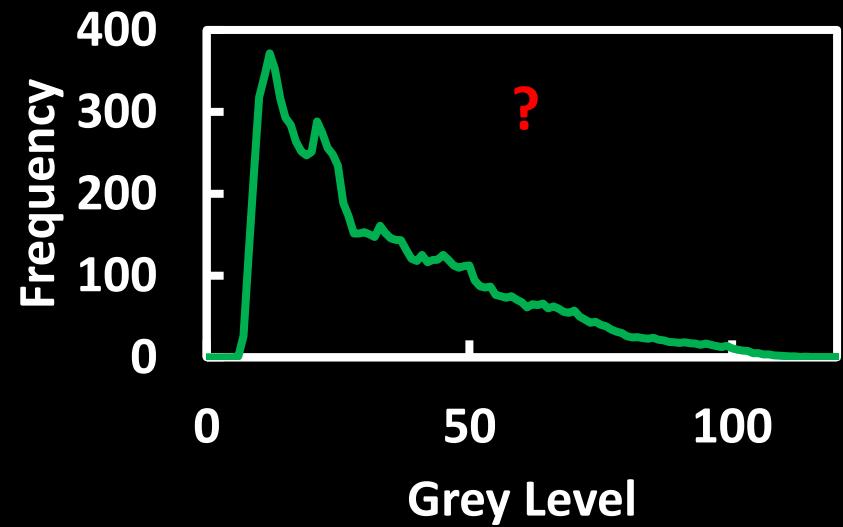
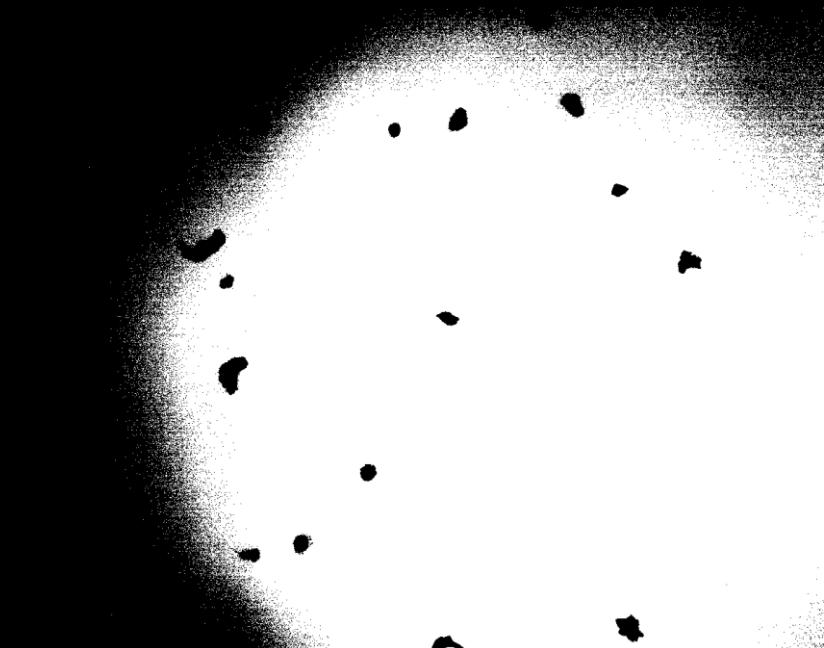
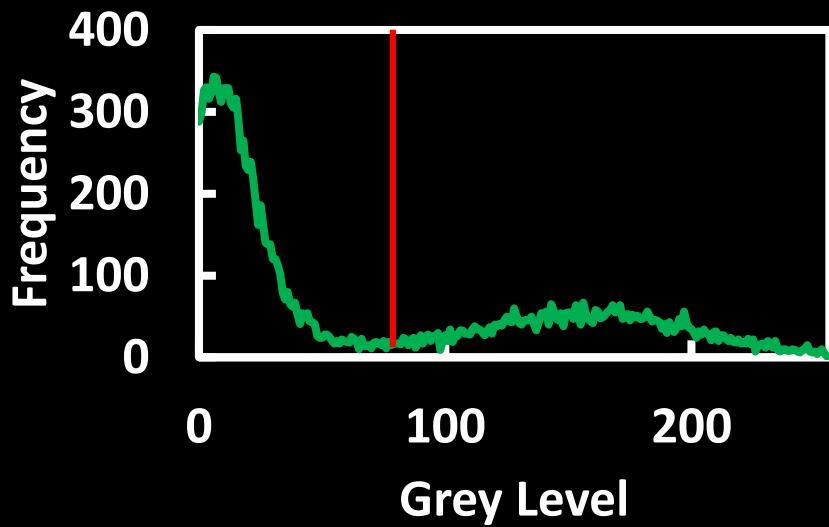
BOUNDARY BETWEEN REGIONS IS AMBIGUOUS



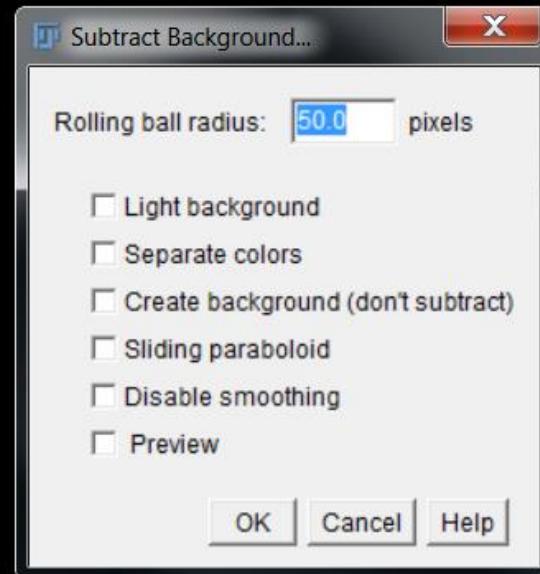
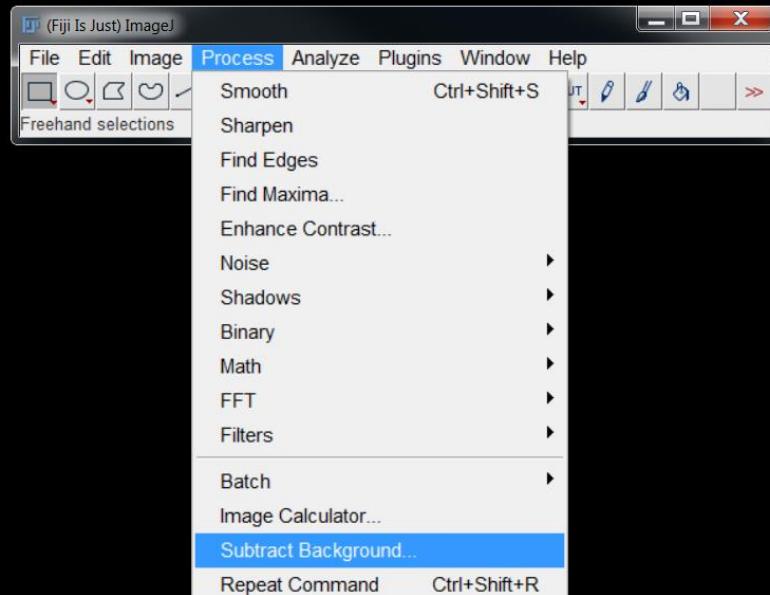
Segmentation is always an estimate



UNEVEN BACKGROUND POSES PROBLEMS

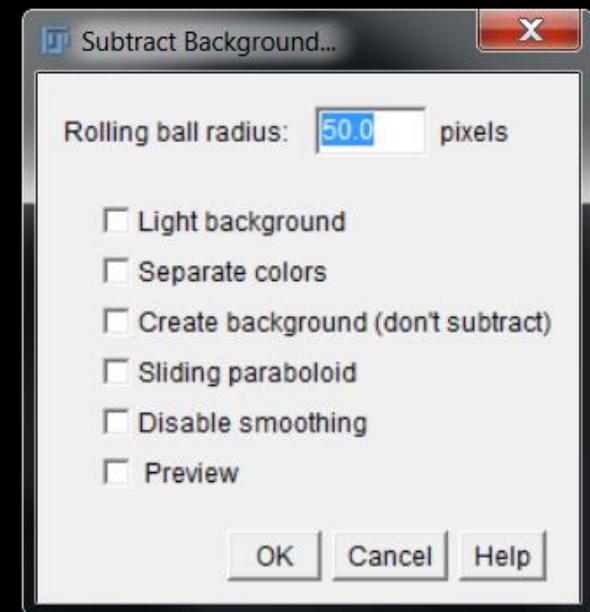


BACKGROUND SUBTRACTION

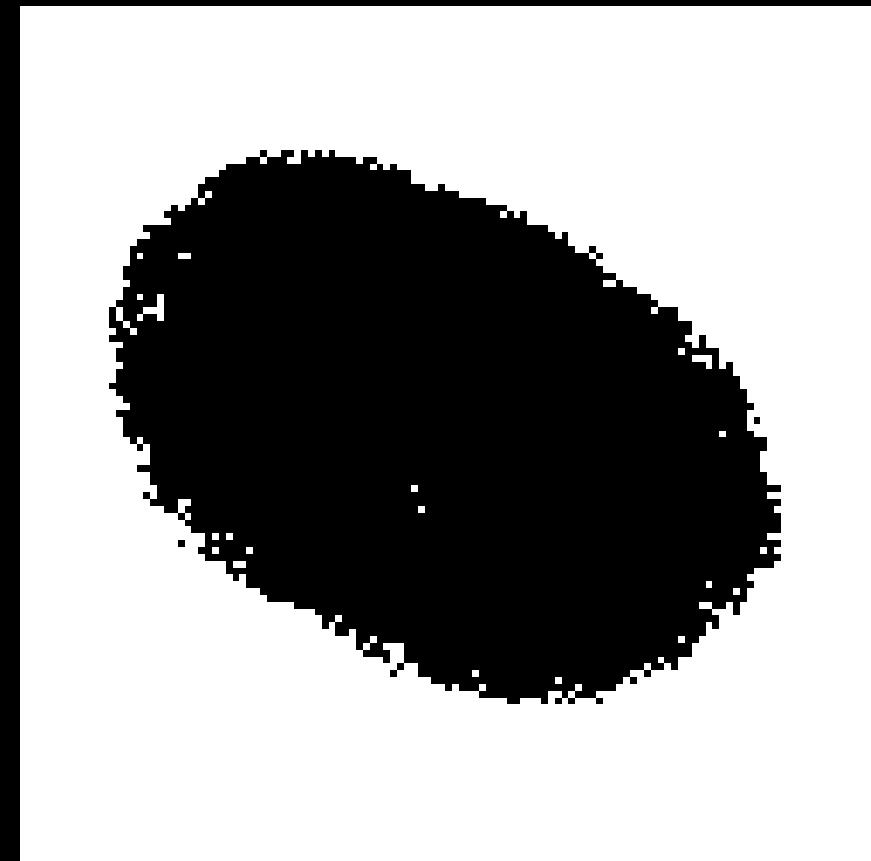


DEMO 4 – CORRECTING UNEVEN BACKGROUND

- Investigate influence of filter radius
- Background subtractor creates an estimate of background and removes from image

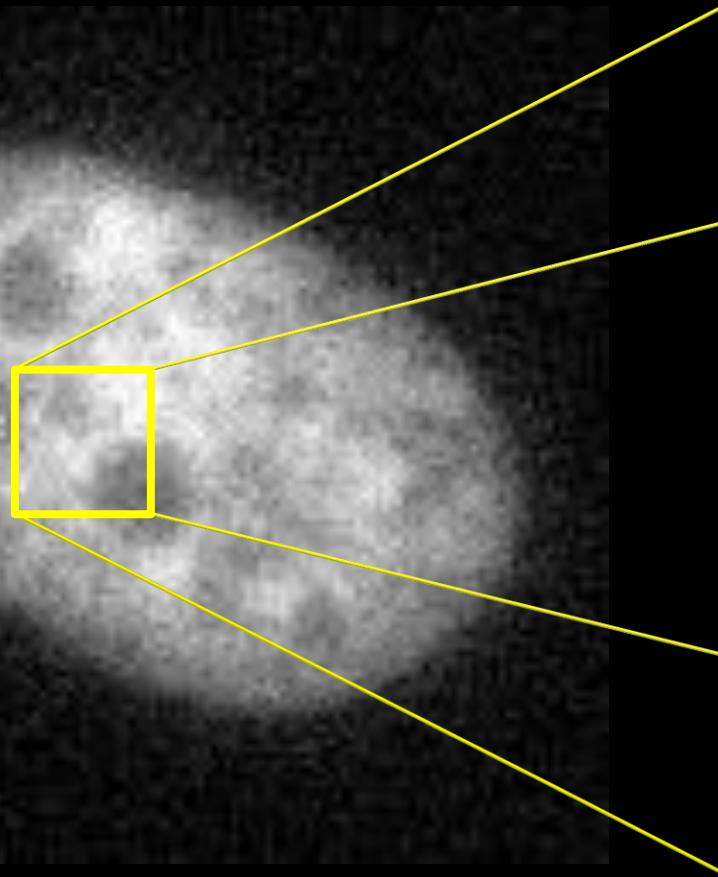


ANOTHER OBSTACLE TO “GOOD” SEGMENTATIONS IS SPECKLE NOISE...



Filtering can be employed to improve segmentation

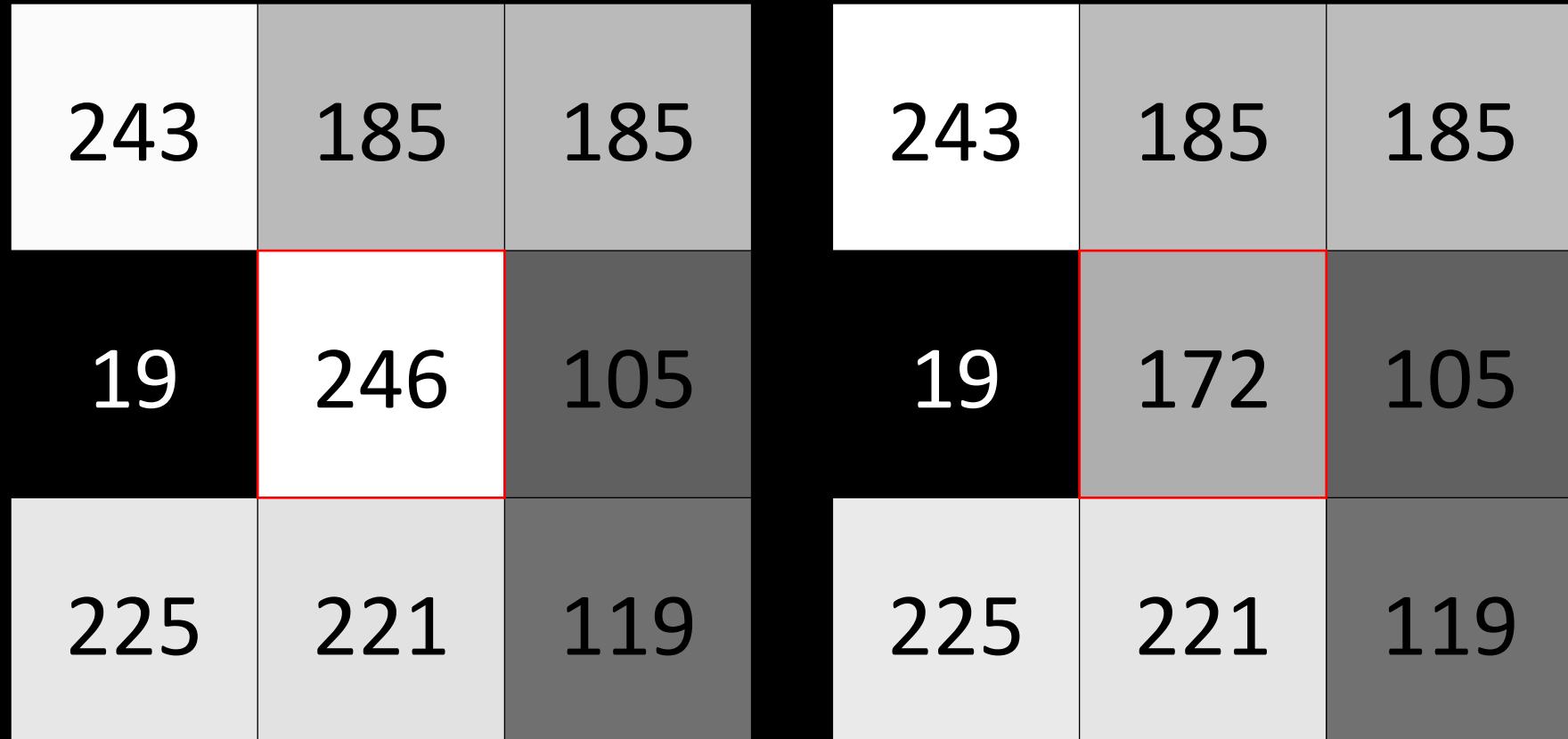
WHAT IS FILTERING?



180	206	167	165	185	181	197	213	231	213	246	230	245	228	245
183	195	163	155	167	172	201	203	234	229	250	245	254	236	247
199	163	162	168	160	184	206	199	207	219	239	231	252	222	238
195	171	162	168	157	170	168	198	224	209	212	205	221	240	252
174	152	141	174	173	157	163	195	229	221	208	202	253	240	250
178	175	181	152	191	193	176	205	202	212	196	192	245	247	249
207	195	178	200	174	196	174	198	188	195	219	205	232	247	214
200	212	217	212	197	183	180	203	229	217	235	215	225	223	242
201	204	212	241	225	199	211	229	220	217	200	201	185	197	182
210	216	226	245	219	213	218	227	206	200	181	159	179	156	165
196	195	212	220	224	234	237	219	192	196	188	186	151	121	149
195	214	198	218	213	229	229	210	166	174	166	161	156	126	127
208	189	206	194	247	234	219	203	164	145	128	131	102	132	137
204	190	201	205	200	227	230	194	153	140	151	112	121	133	141

- Generally refers to simple arithmetic operations on small numbers of pixels

MEAN/AVERAGING FILTERING



Replaces central value with average of all values

FILTERING IN ACTION

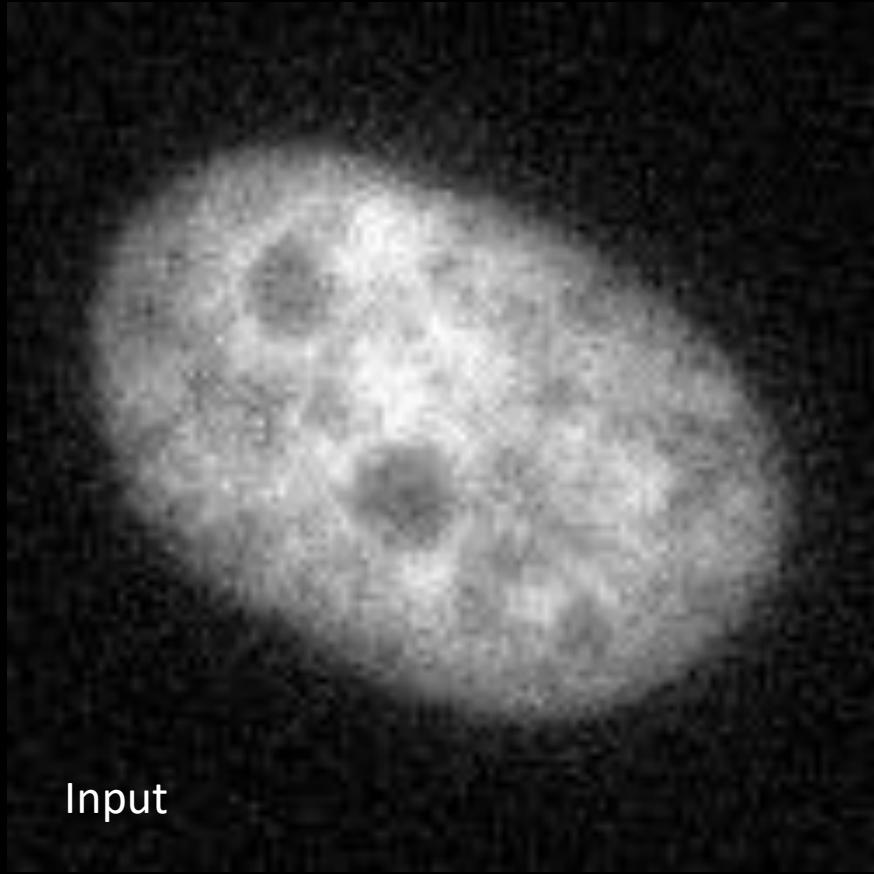
168	186	192	197	199	202	201	196	196	202	206	208	214	228	224	220	223	212	206	205
177	196	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216
184	193	194	198	203	225	231	227	224	223	221	217	201	181	178	168	161	167	160	163
180	193	200	196	198	221	225	224	227	224	212	196	180	170	166	151	146	142	138	148
191	192	188	197	205	214	226	226	234	228	199	177	169	160	148	141	133	132	132	136
202	200	196	197	202	210	220	222	228	230	201	169	160	156	143	131	127	130	125	131
214	203	196	200	208	199	210	210	217	209	193	164	153	149	135	127	128	130	119	124
217	204	198	204	198	195	190	192	194	187	175	153	142	137	130	120	128	125	117	122
208	198	189	196	201	188	180	180	179	175	163	144	140	129	120	121	117	116	114	115
198	191	187	184	186	181	182	184	180	166	159	145	133	126	117	118	116	113	112	108
184	180	180	178	184	182	189	192	202	180	152	138	130	119	117	110	117	112	115	113
174	175	175	175	180	178	189	199	209	193	167	142	136	130	121	116	116	113	116	112
167	169	171	174	176	177	173	192	204	212	184	162	146	144	137	129	126	124	116	113
163	171	173	171	182	179	179	186	196	205	188	177	161	150	148	139	128	122	119	119
167	179	177	175	177	174	180	184	190	194	194	182	173	162	150	145	134	132	132	130
172	185	186	183	176	175	171	172	180	185	184	183	179	161	148	143	142	148	141	141
178	184	188	181	175	169	167	170	173	173	174	167	168	169	164	159	157	160	156	161
174	180	183	174	170	161	163	167	164	169	161	161	163	175	180	179	176	181	191	194
169	170	168	167	159	160	159	165	170	164	161	160	171	177	189	185	182	190	203	205
160	157	153	154	157	158	164	163	169	166	160	161	167	178	185	181	183	190	209	217

Input

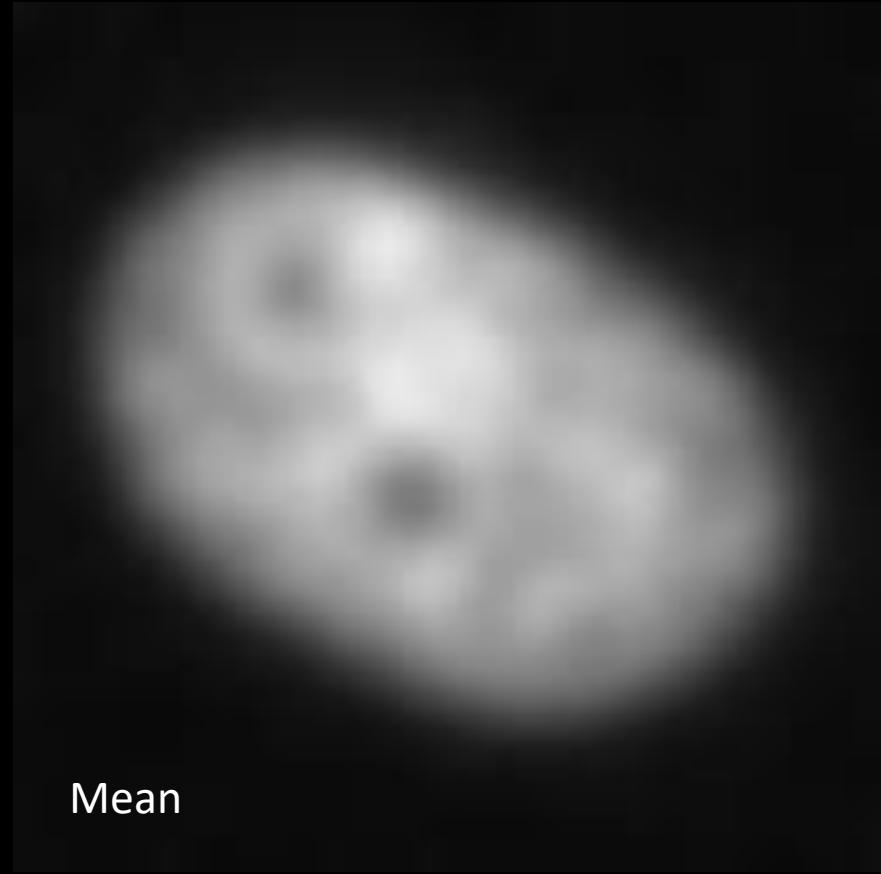
182	185	194	197	203	208	210	208	206	207	210	213	215	215	212	209	205	201	197	195
184	187	194	197	205	212	216	215	212	212	214	213	210	205	200	196	191	188	185	184
187	190	196	198	207	216	224	224	221	219	215	208	198	189	179	173	167	164	164	163
189	191	195	198	206	216	224	227	226	221	211	197	183	173	163	155	149	146	146	146
193	194	195	198	204	213	221	226	227	220	204	185	171	161	152	143	137	134	135	135
200	198	197	199	204	210	215	221	223	215	197	176	162	153	143	135	131	128	129	128
207	203	200	200	201	204	205	209	210	204	187	168	154	145	136	130	127	125	125	123
207	203	199	199	199	197	194	195	194	188	174	159	146	137	130	125	124	122	120	119
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193	191	187	187	187	187	186	184	185	182	173	158	145	134	126	120	117	116	115	113
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169	171	173	175	175	176	176	180	187	196	196	189	174	162	152	145	137	131	126	122
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178	180	182	180	176	174	174	174	174	176	180	183	182	178	172	164	156	149	147	144
179	181	183	180	174	170	168	170	173	174	173	171	170	167	164	161	161	161	164	164
176	177	177	177	174	168	165	165	166	168	168	166	165	168	173	175	175	174	177	182
168	168	167	165	162	161	162	165	166	166	165	163	163	168	176	181	182	183	189	198
164	163	162	160	159	160	162	165	166	165	162	163	169	178	183	184	185	193	202	209

Output

MEAN FILTERING “BLURS” NOISE



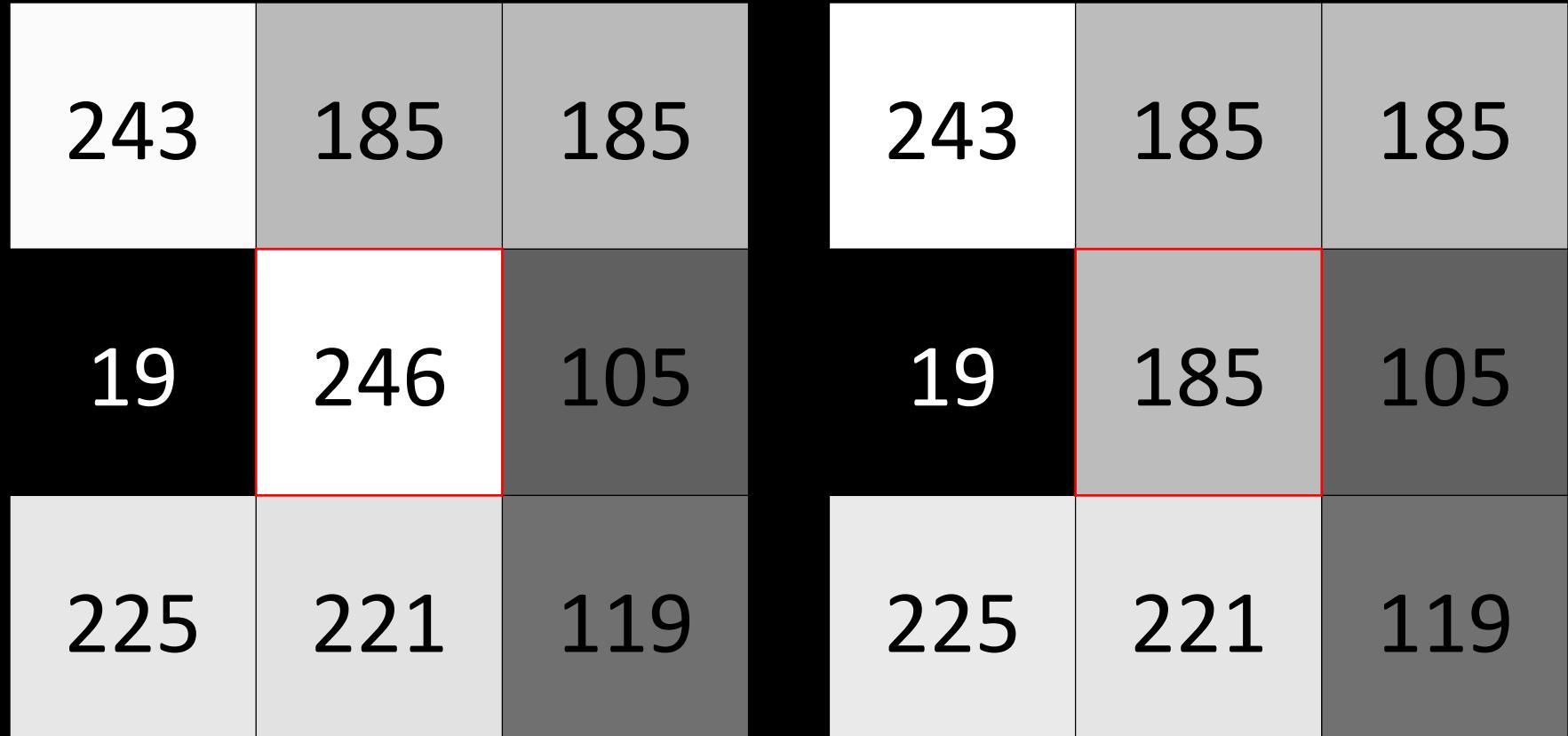
Input



Mean

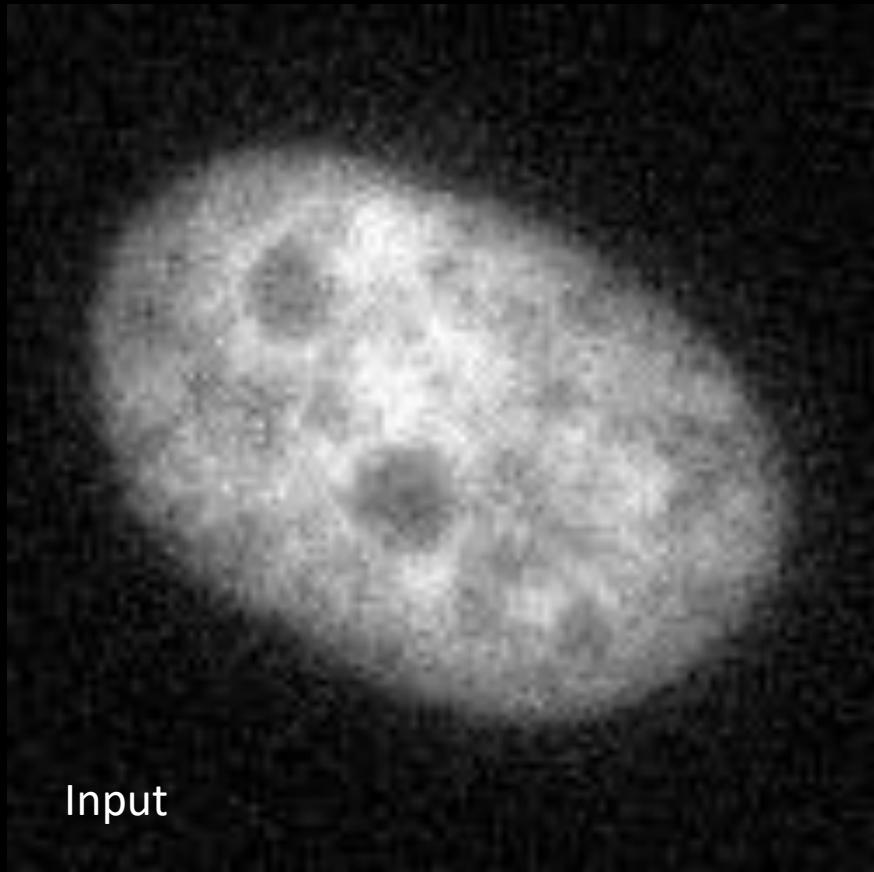
But remember, information is also lost!

MEDIAN FILTERING



Replaces central value with median value

MEDIAN FILTERING PRESERVES EDGES

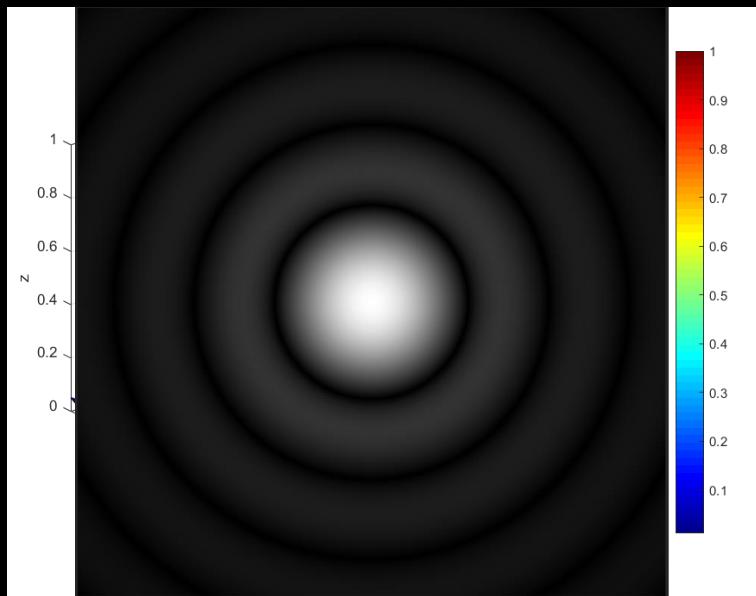


Input

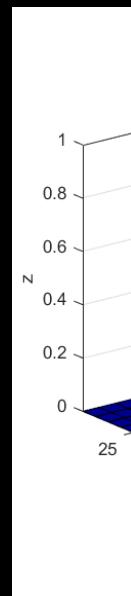


Median

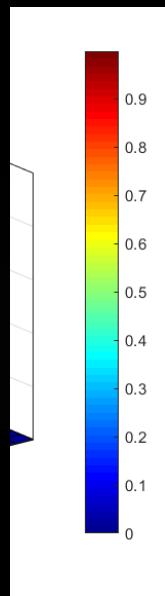
GAUSSIAN FILTERING



Airy Disk



2D Gaussian



1.00	1.00	1.00
1.00	1.00	1.00
1.00	1.00	1.00

Gaussian Filter

- Approximation of Airy Disk
- Essentially a weighted mean filter

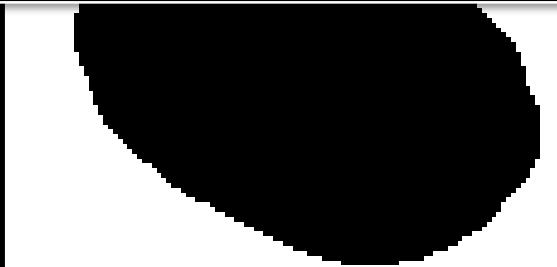
Processing:



Unfiltered Output



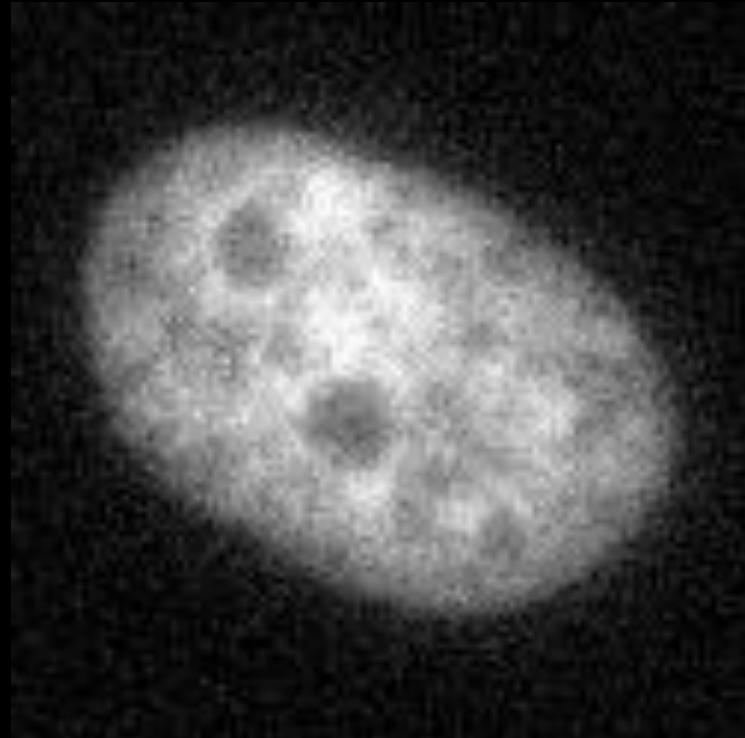
Median



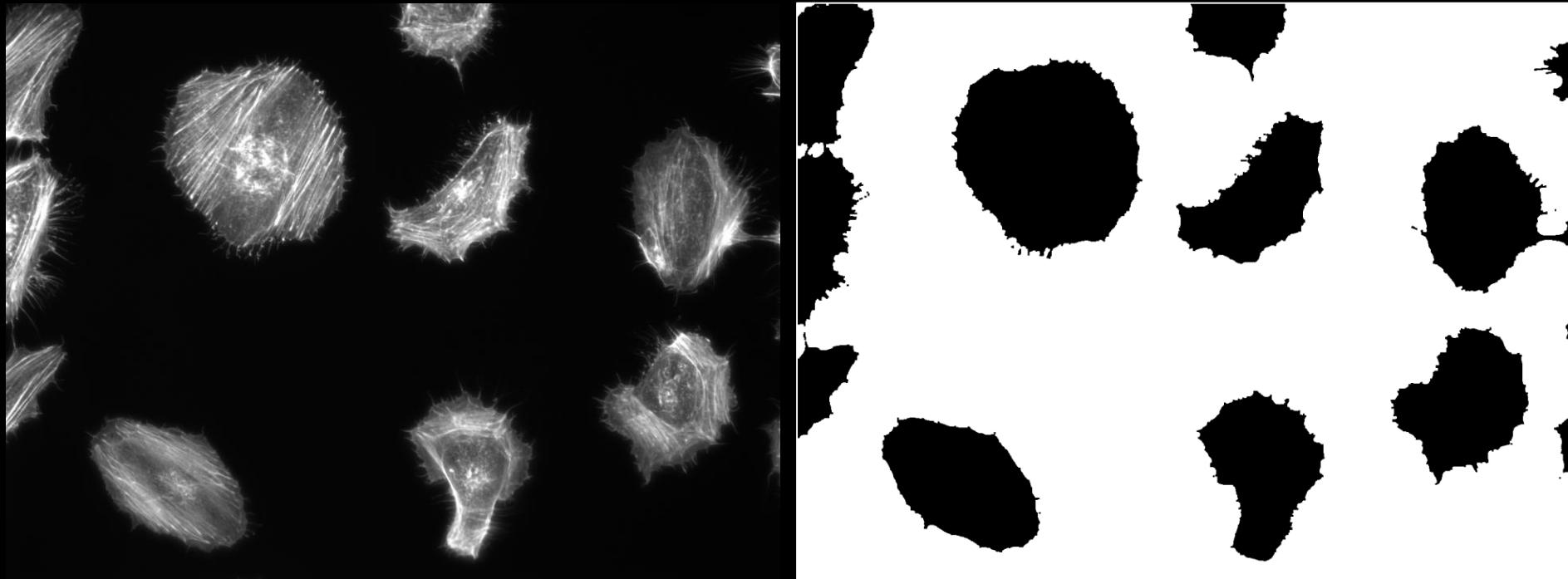
Filtered Output

DEMO 5 - FILTERS

- Notice the effect of varying filter radius
- Observe the difference in edge preservation between mean and median filters

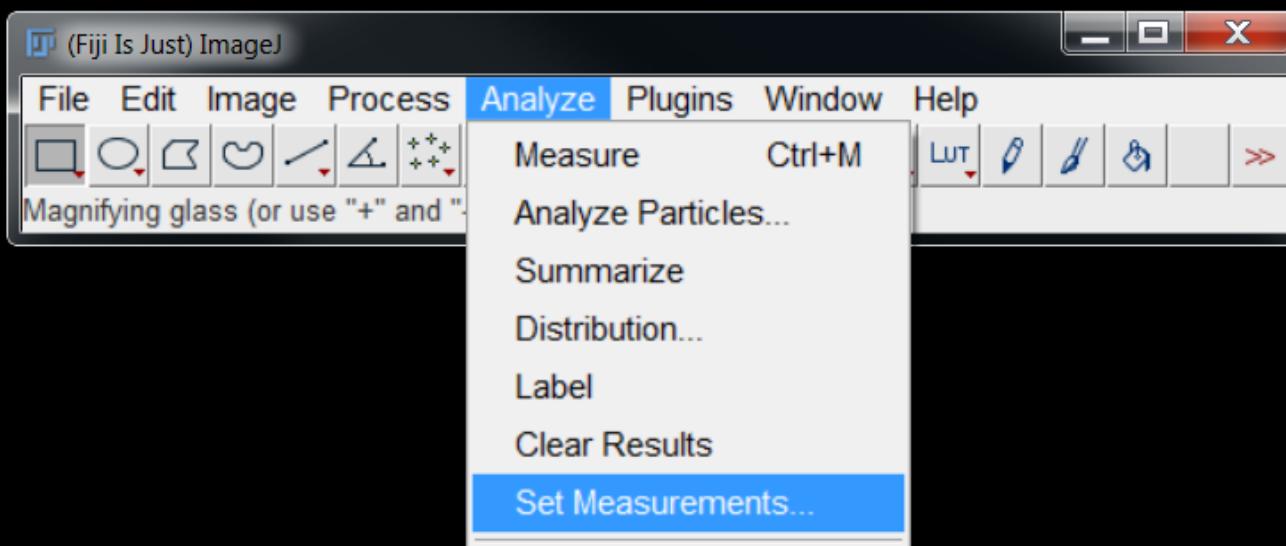
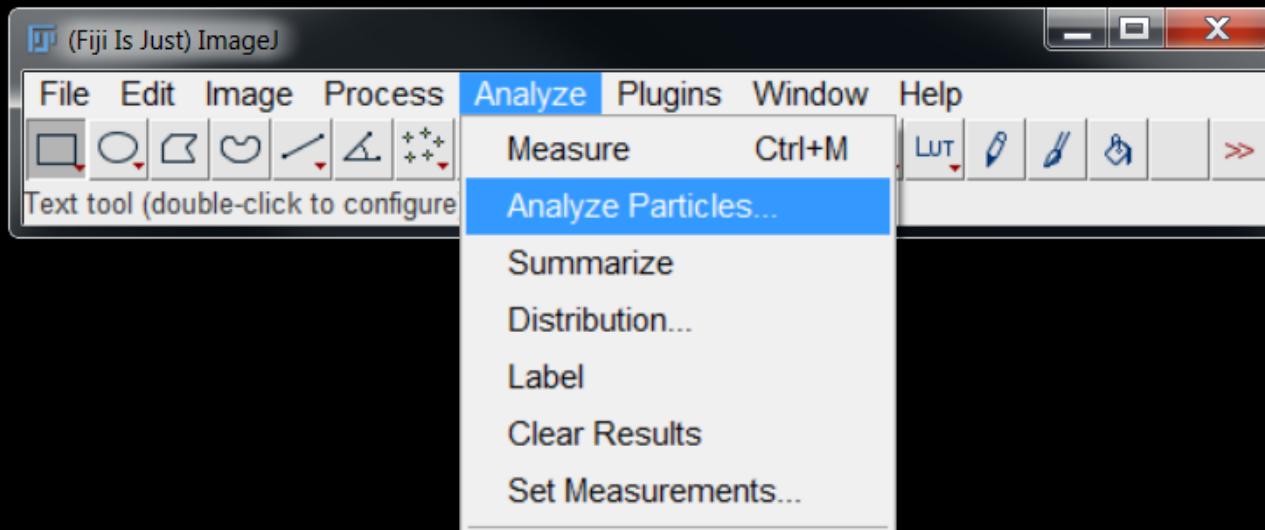


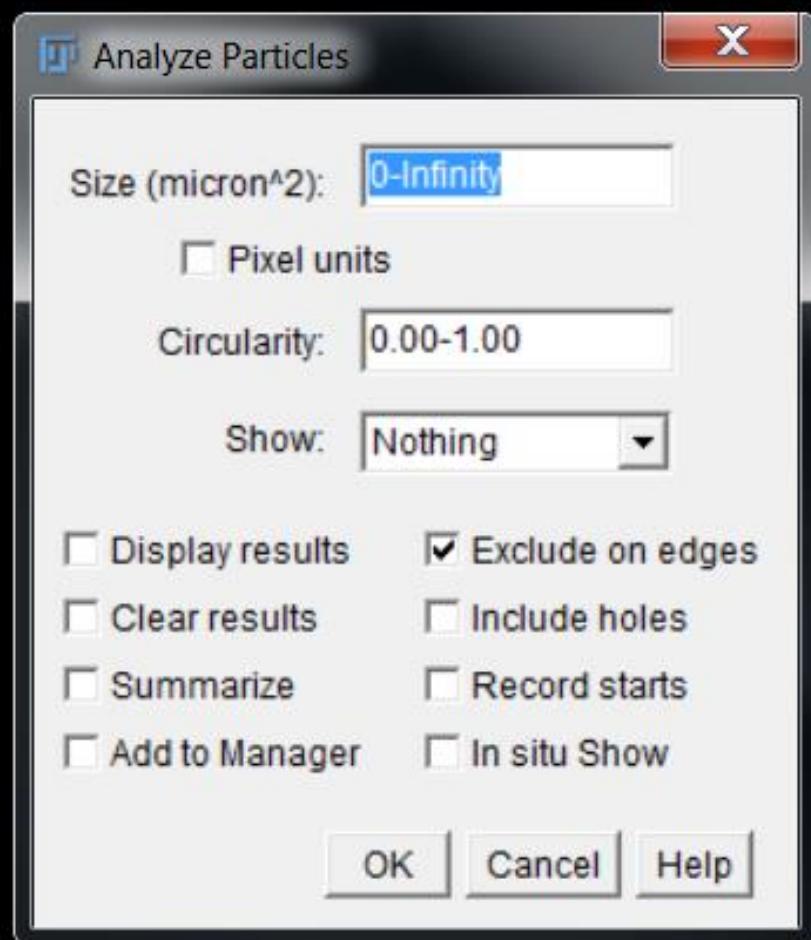
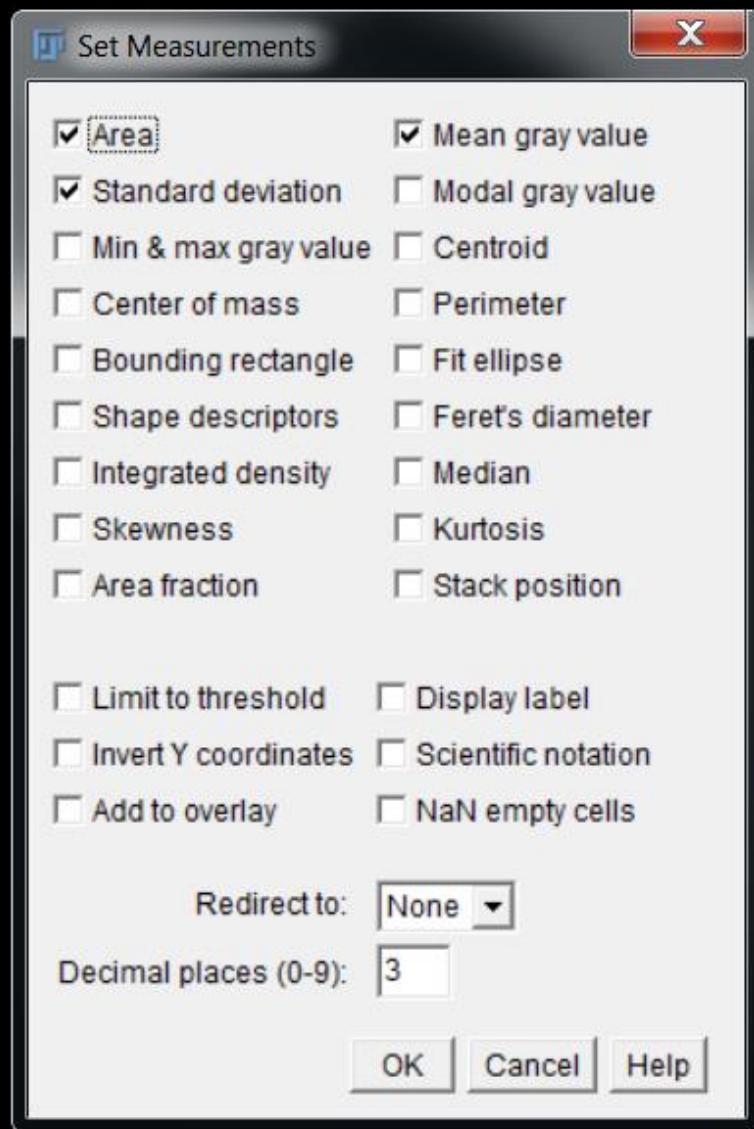
MORPHOLOGICAL ANALYSIS

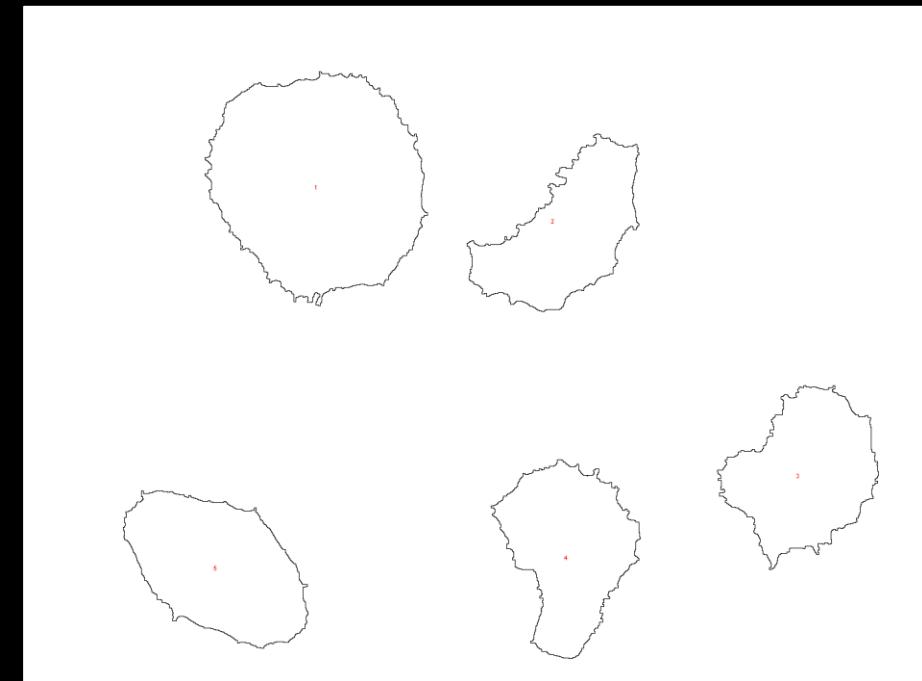
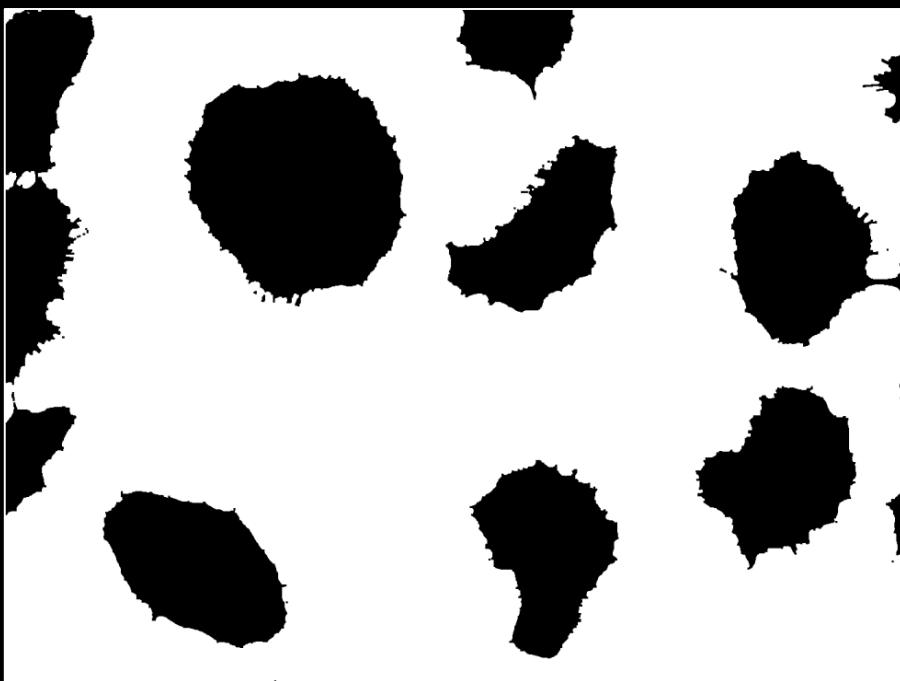


How do we extract numerical data from segmented images?

MORPHOLOGICAL ANALYSIS



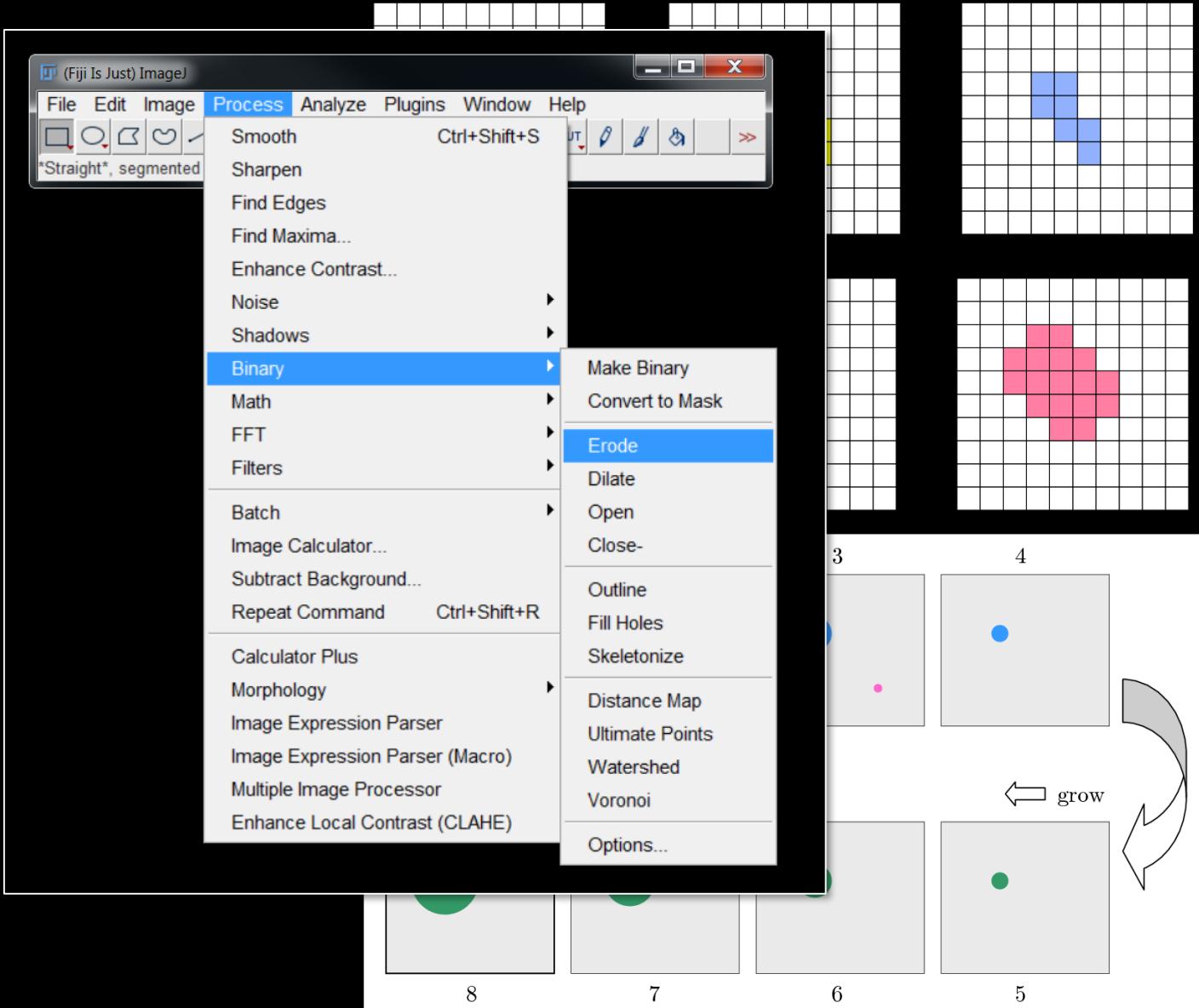




	Results																				
	File	Edit	Font	Results																	
	Area	X	Y	XM	YM	Perim.	BX	BY	Width	Height	Major	Minor	Angle	Circ.	Feret	IntDen	RawIntDen	FeretX	FeretY	FeretAngle	MinFer
1	88461	452.335	269.404	452.335	269.404	1385.894	277	99	343	360	351.863	320.102	126.303	0.579	371.602	22557555	22557555	311	146	130.635	324.8
2	37578	833.182	346.780	833.182	346.780	1097.744	681	195	265	273	311.118	153.787	46.201	0.392	332.182	9582390	9582390	684	419	38.768	181.8
3	41450	1201.315	717.108	1201.315	717.108	1062.590	1067	582	248	283	260.470	202.617	55.433	0.461	300.483	10569750	10569750	1147	865	70.359	216.0
4	40950	837.361	834.134	837.361	834.134	1044.815	718	696	230	306	281.859	184.983	95.828	0.471	306.863	10442250	10442250	820	696	94.298	213.8
5	45631	296.057	861.770	296.057	861.770	949.034	151	744	285	242	316.443	183.601	145.191	0.637	329.509	11635905	11635905	155	776	146.889	206.4

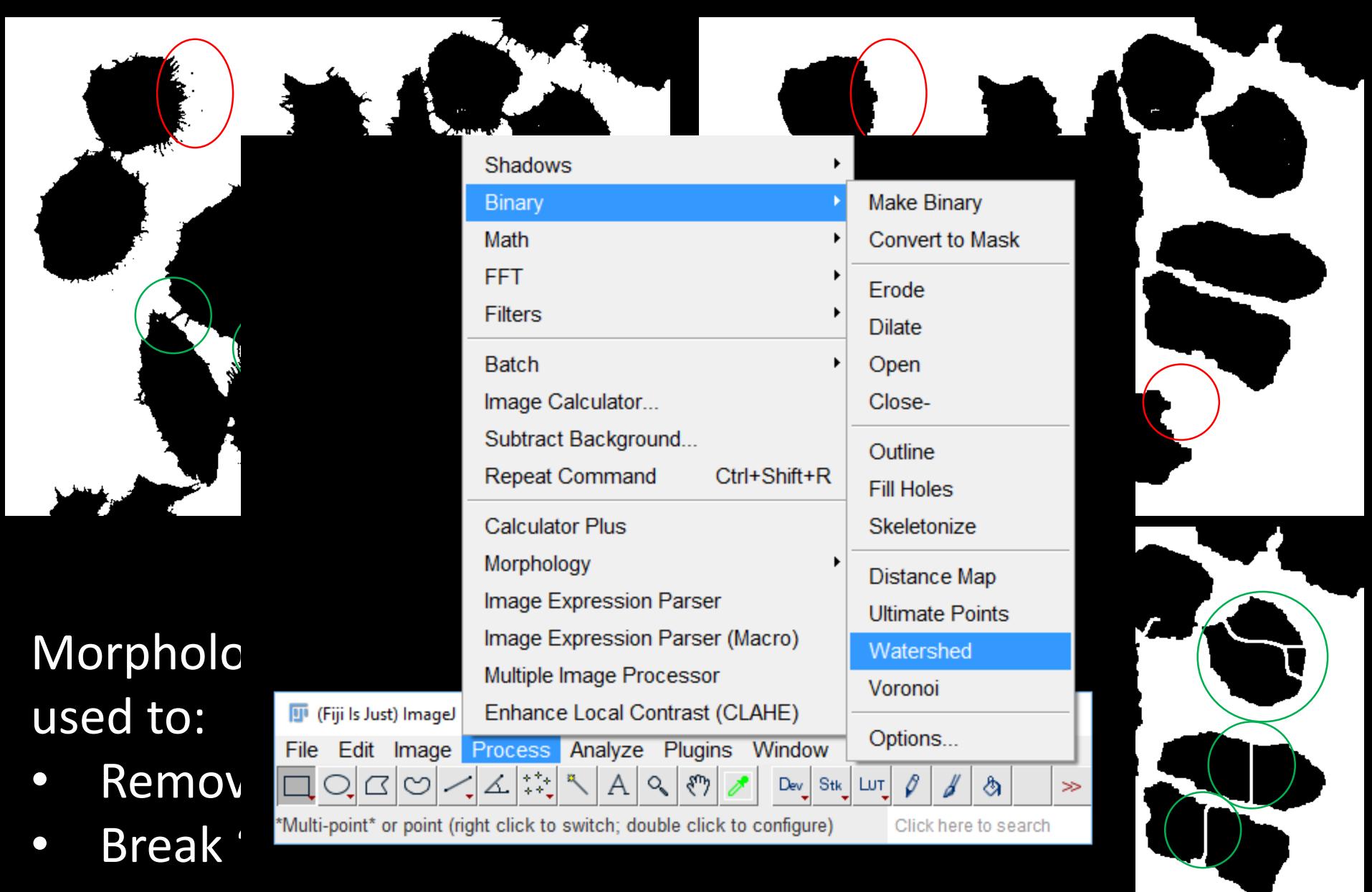
MORPHOLOGICAL FILTERING

Erosion



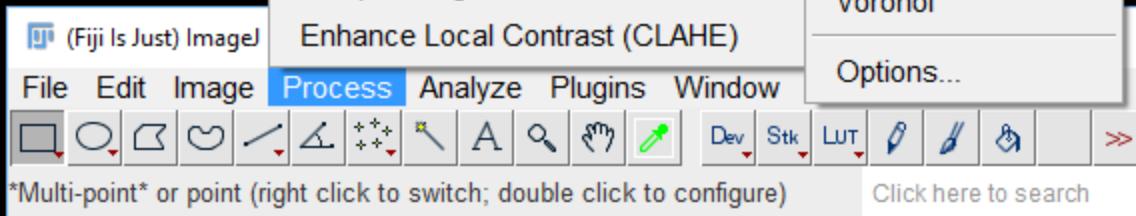
Dilation

Erosion + Dilatation



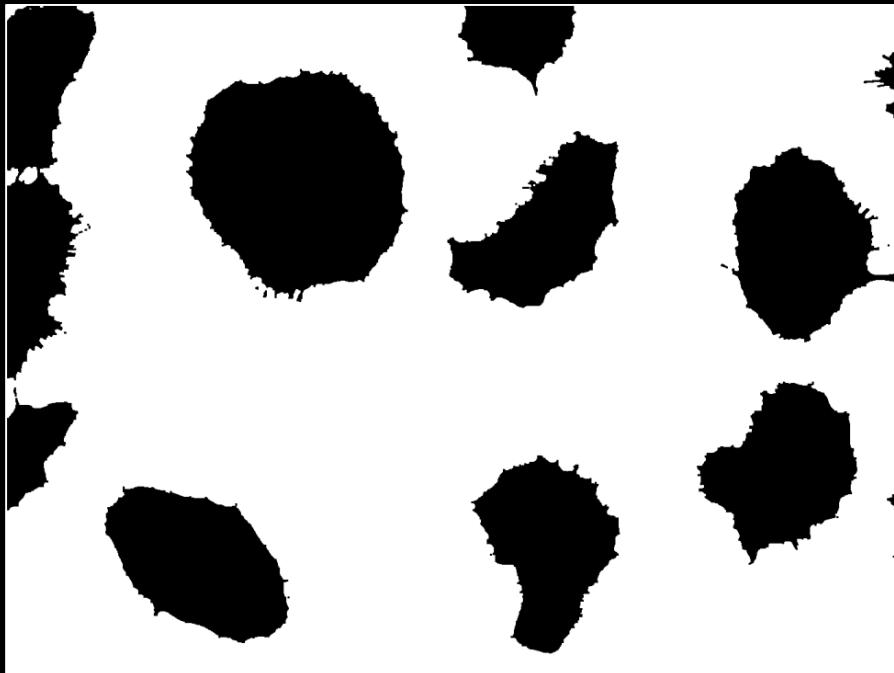
Morphology
used to:

- Remove noise
- Break boundaries
- Watershed: break apart touching cells

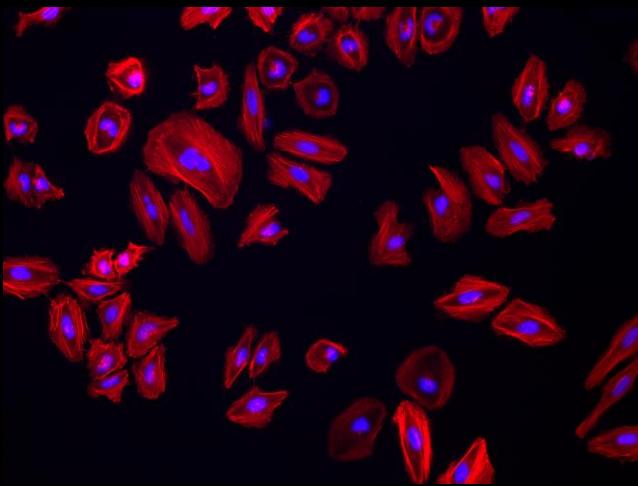


DEMO 6 – WORKING WITH BINARY IMAGES

- Demo particle analyser and morphological filters
- Show how to specify background colour for analyser



CHALLENGE 1



1. Count the number of cells
2. Estimate the mean cell area

Note: You may find it helpful to duplicate and/or rename images as you work

Filter Noise

- Process > Filters

Segment Cells From Background

- Image > Adjust > Threshold

Manipulate Segmentation

- Process > Binary

Specify Morphological Measurements

- Analyze > Set Measurements

Quantify Morphology

- Analyze > Analyze Particles

CHALLENGE 1

Filter Noise

- Process > Filters

Segment Cells From Background

- Image > Adjust > Threshold

Manipulate Segmentation

- Process > Binary

Specify Morphological Measurements

- Analyze > Set Measurements

Quantify Morphology

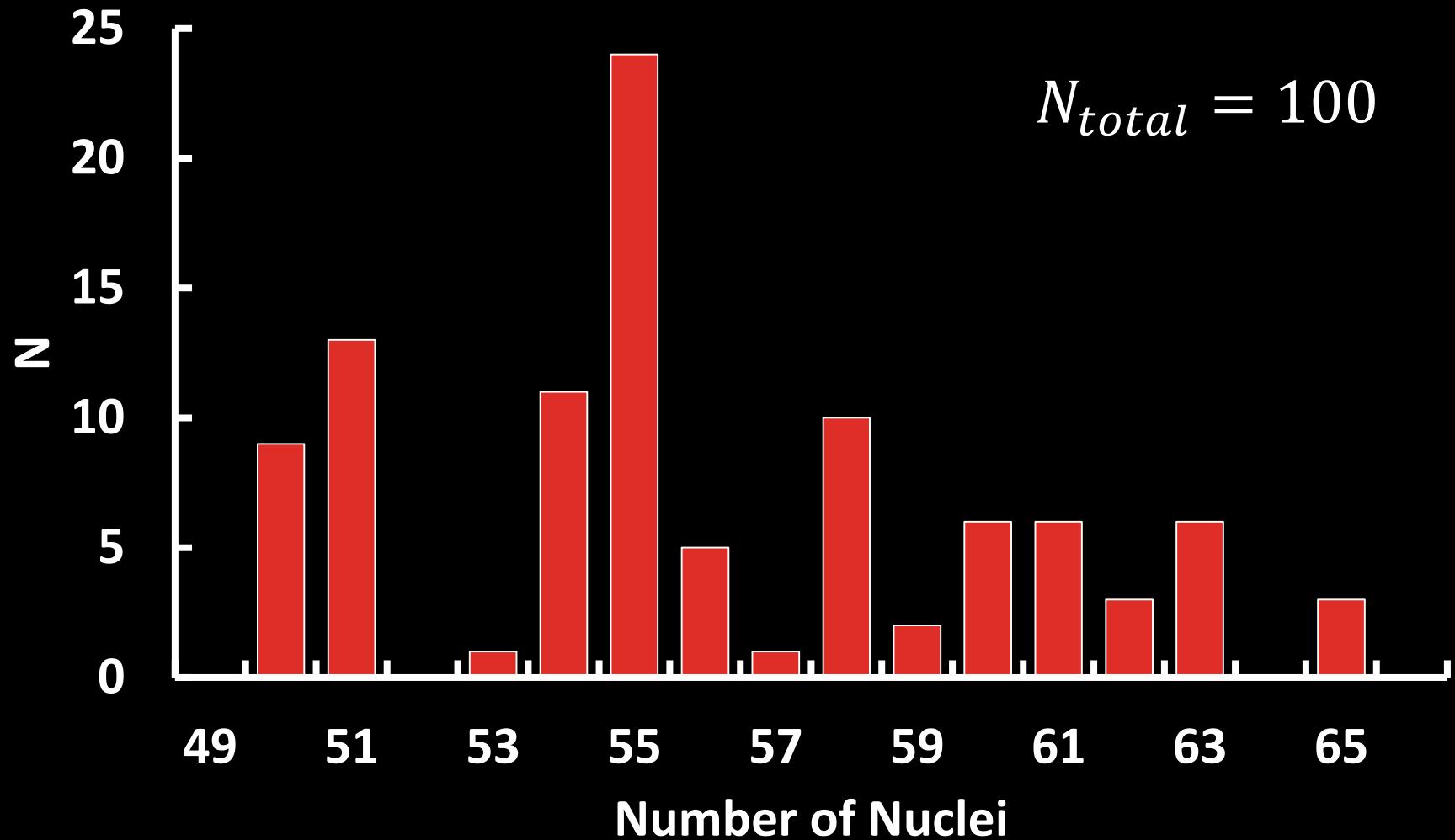
- Analyze > Analyze Particles

- Which variables in your analysis pipeline will have the greatest impact on your result?
- Could the images be improved in any way to facilitate easier analysis?
- Can you think of an alternative approach?

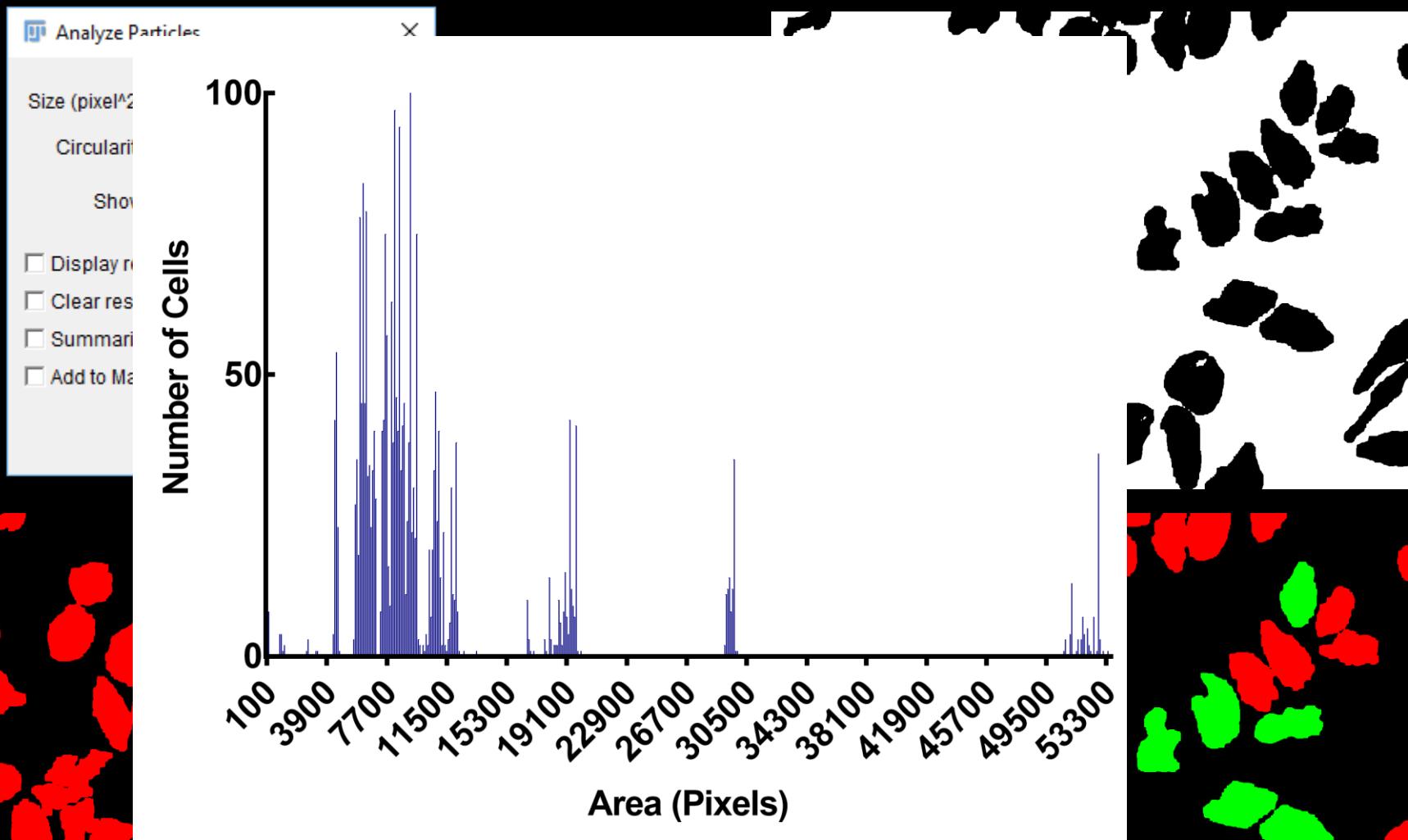
SOLUTION TO CHALLENGE 1



HOW DO PARAMETER VALUES AFFECT THE RESULT?



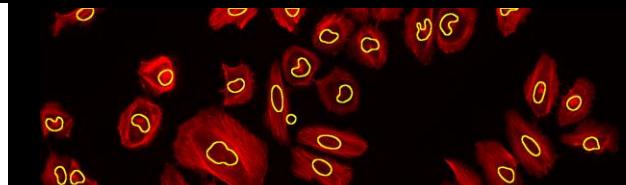
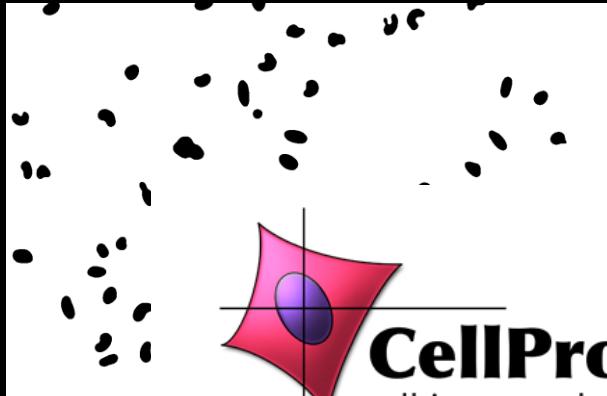
USING MORPHOLOGICAL CRITERIA



REPEAT ANALYSIS ON CLUMPED CELLS



ALTERNATIVE APPROACH: REGION-GROWING



Use nuclei as seeds

 **CellProfiler**
cell image analysis software



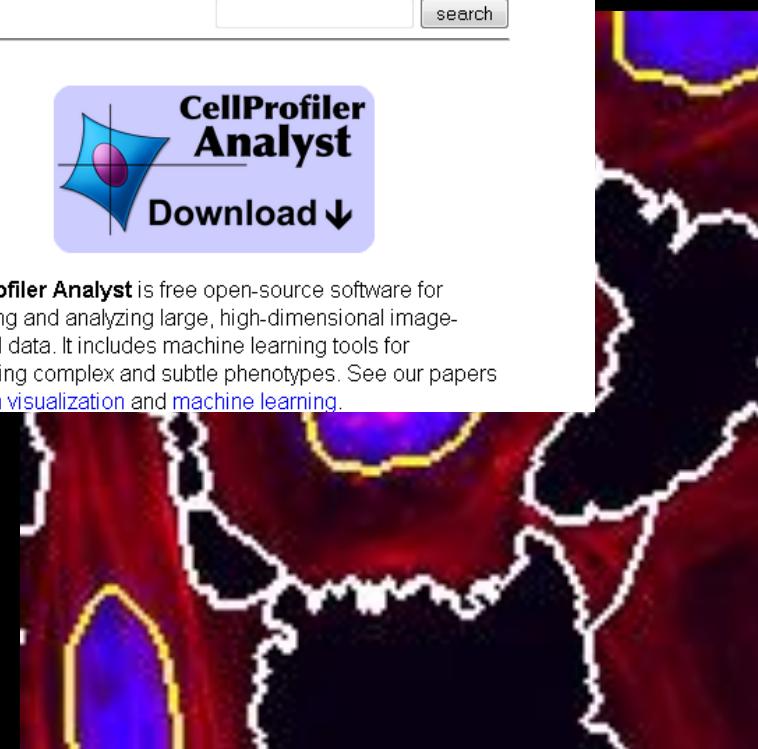
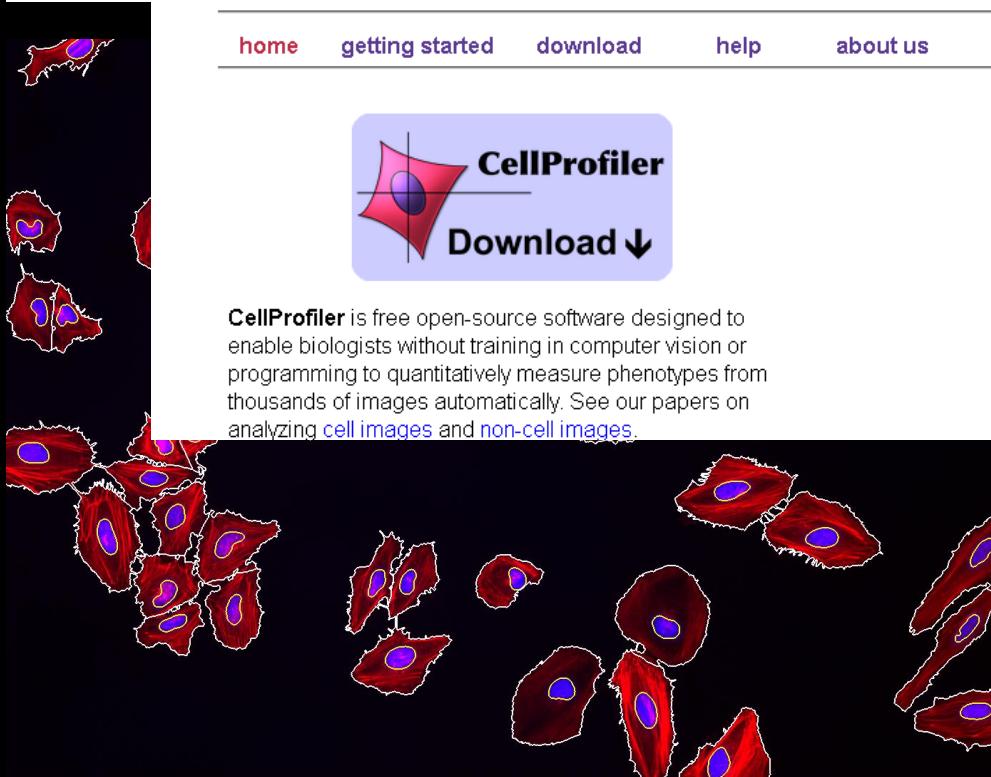
[home](#) [getting started](#) [download](#) [help](#) [about us](#)

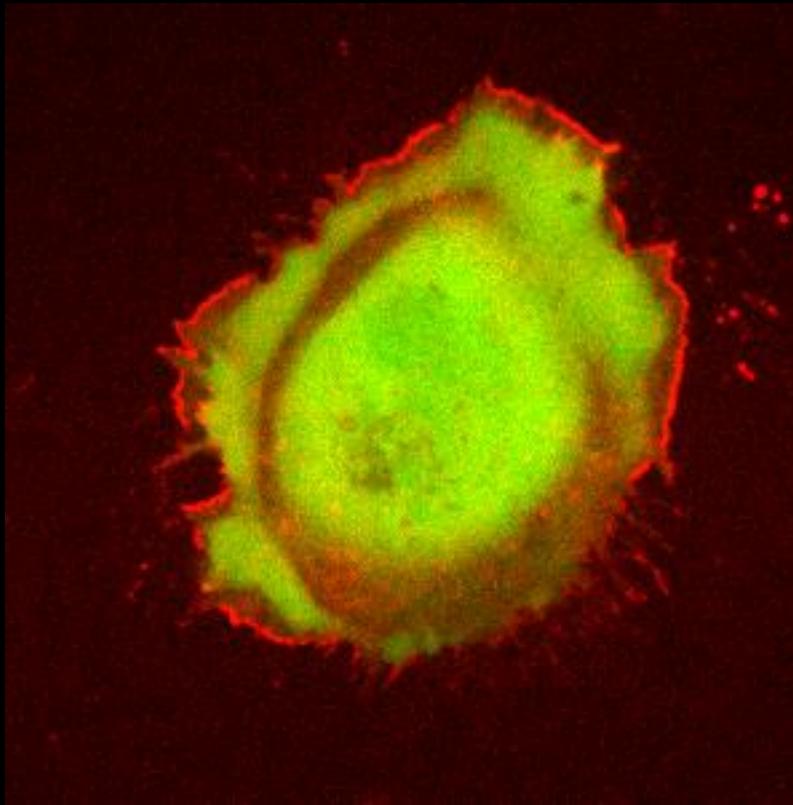
CellProfiler is free open-source software designed to enable biologists without training in computer vision or programming to quantitatively measure phenotypes from thousands of images automatically. See our papers on analyzing [cell images](#) and [non-cell images](#).



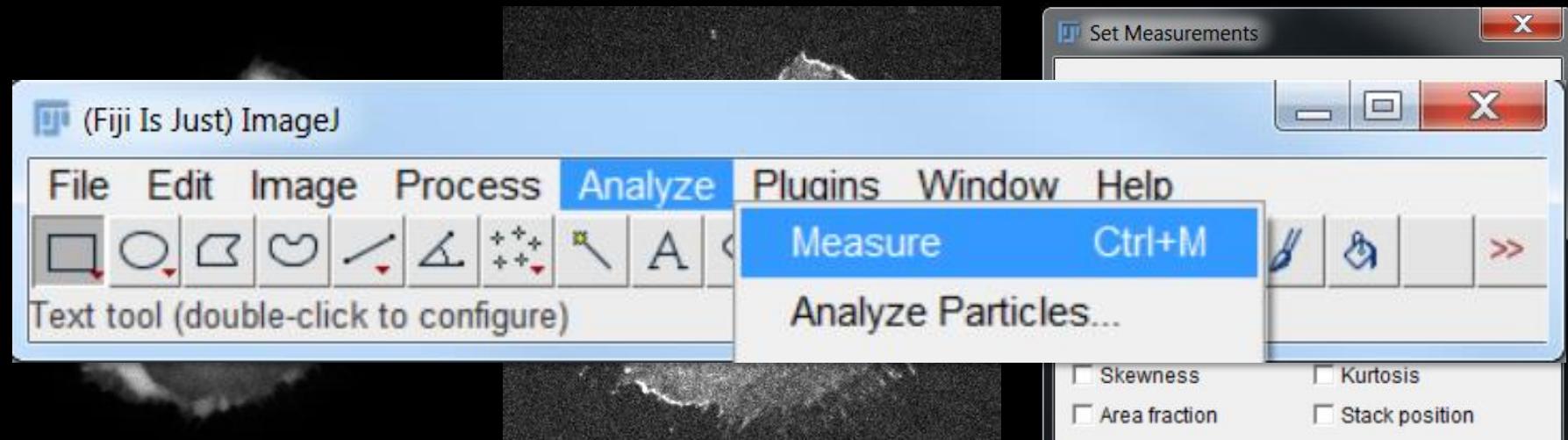
CellProfiler Analyst is free open-source software for exploring and analyzing large, high-dimensional image-derived data. It includes machine learning tools for identifying complex and subtle phenotypes. See our papers on [data visualization](#) and [machine learning](#).



ANALYSING MULTIPLE CHANNELS



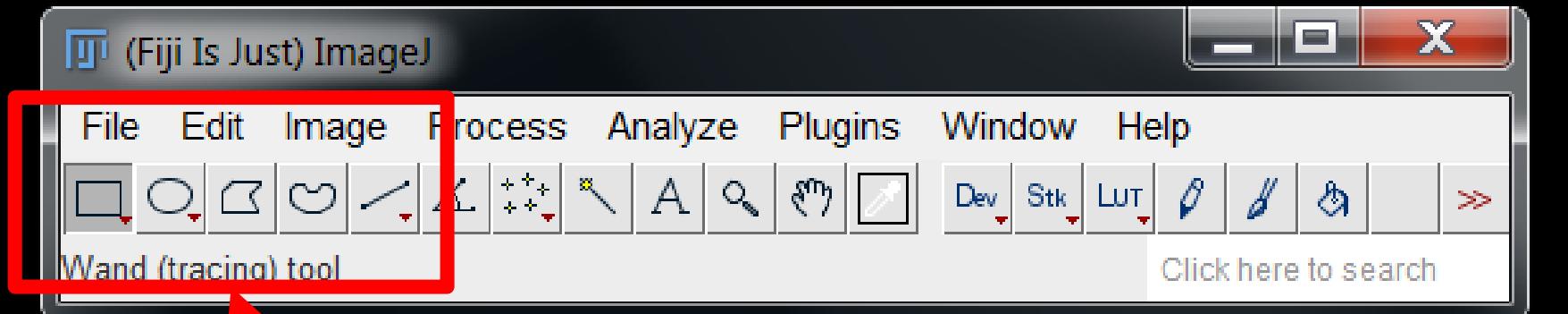
What if we want to analyse protein expression?



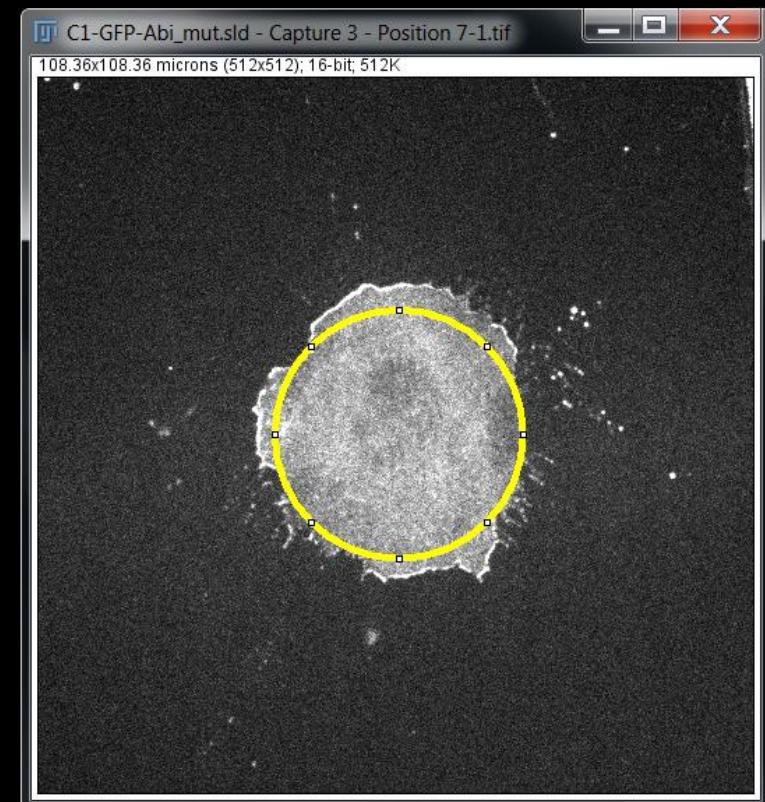
FIJI restricts measurements to within Regions of Interest (ROIs)

1. Generate an ROI
2. Apply ROI to image
3. Measure the pixel values in the image within the ROI

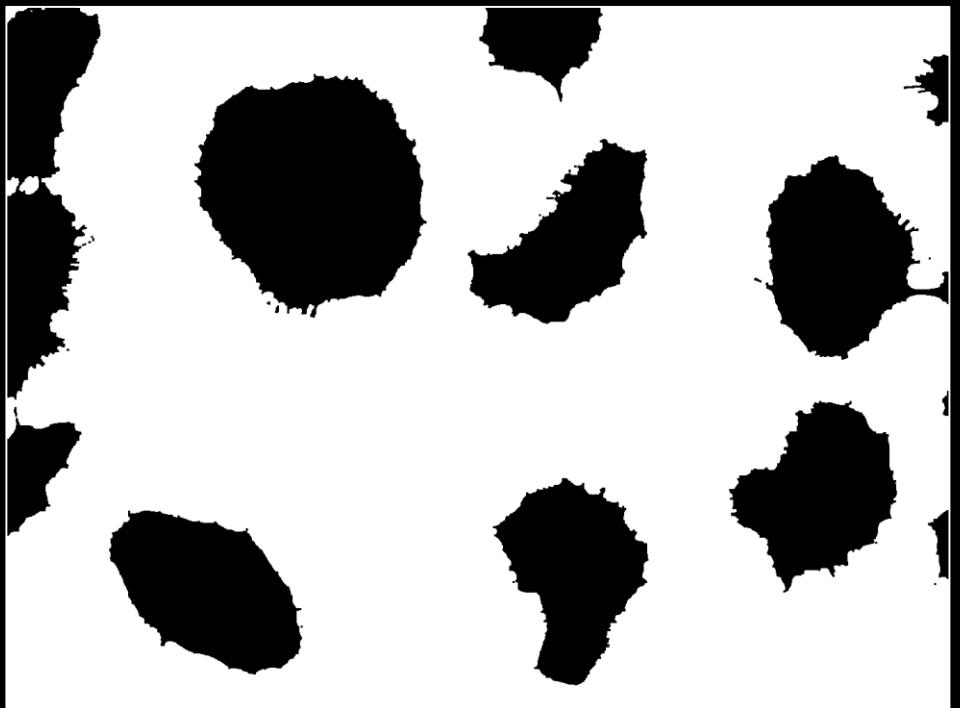
IT'S POSSIBLE TO SPECIFY ROIS MANUALLY



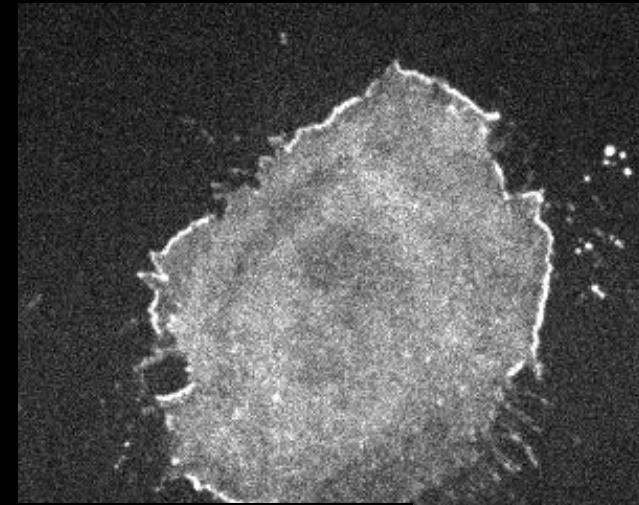
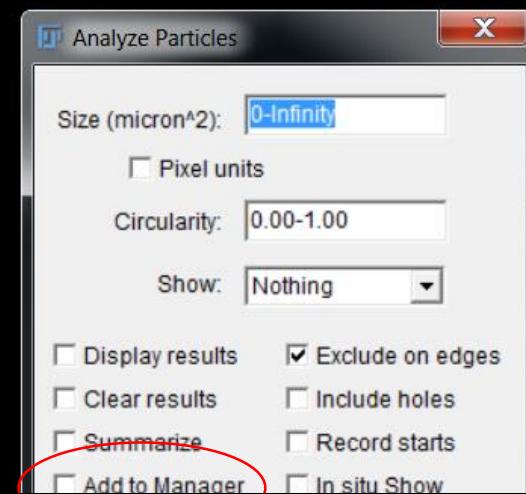
ROI Drawing Tools



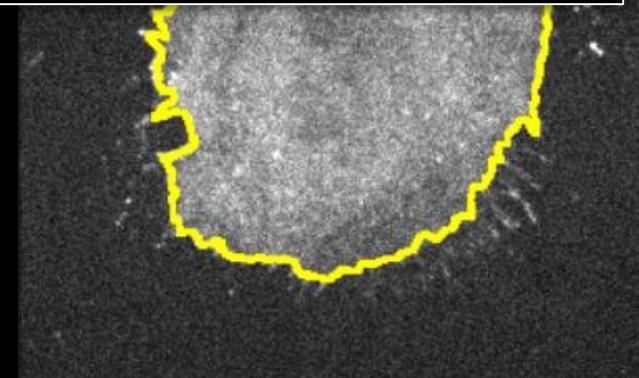
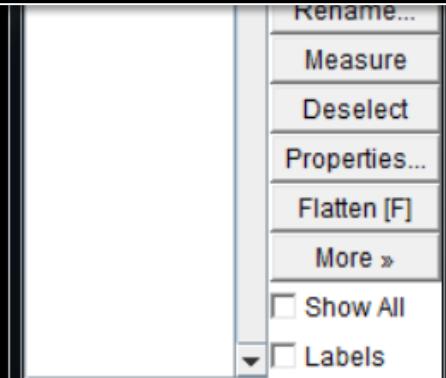
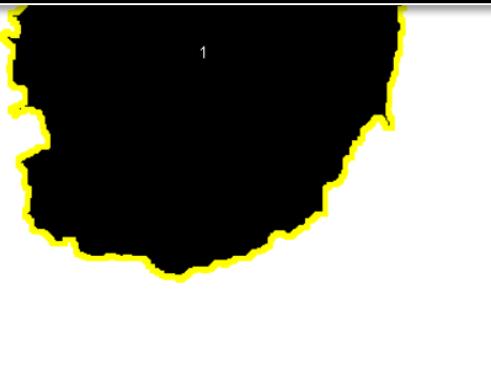
PARTICLE ANALYSER GENERATES ROIS “BEHIND THE SCENES”



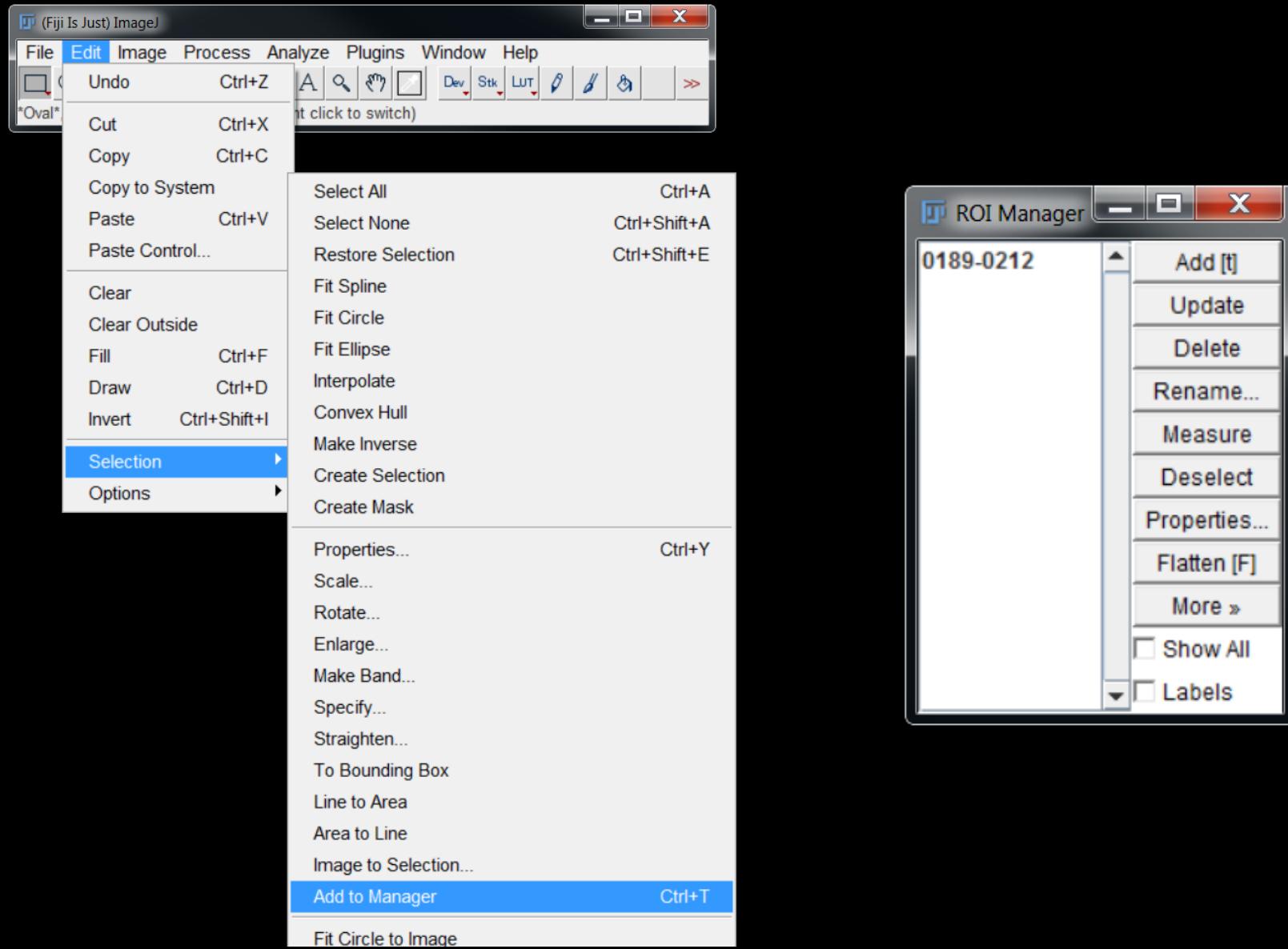
Masks and ROIs can be considered interchangeable



1. Using Particle Analyser to generate an ROI
2. Apply ROI to image
3. Measure the pixel values in the image within the ROI



ROI MANAGER CAN ALSO BE ACCESSED MANUALLY...



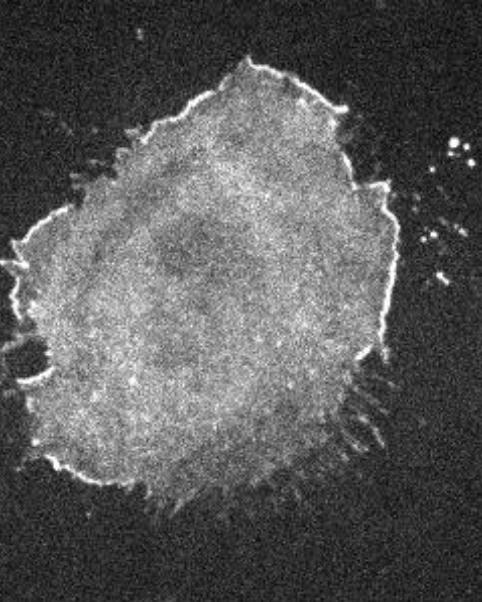
DEMO 7 – GENERATING ROIS AUTOMATICALLY

Demonstration of Particle Analyser's ability to generate regions of interest

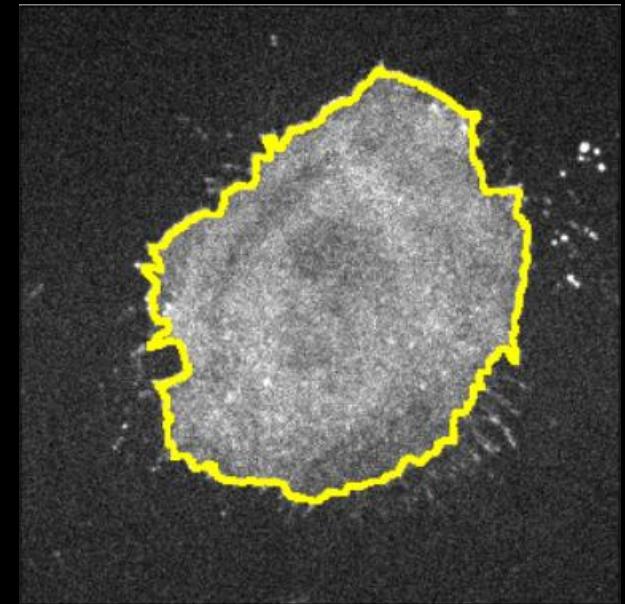
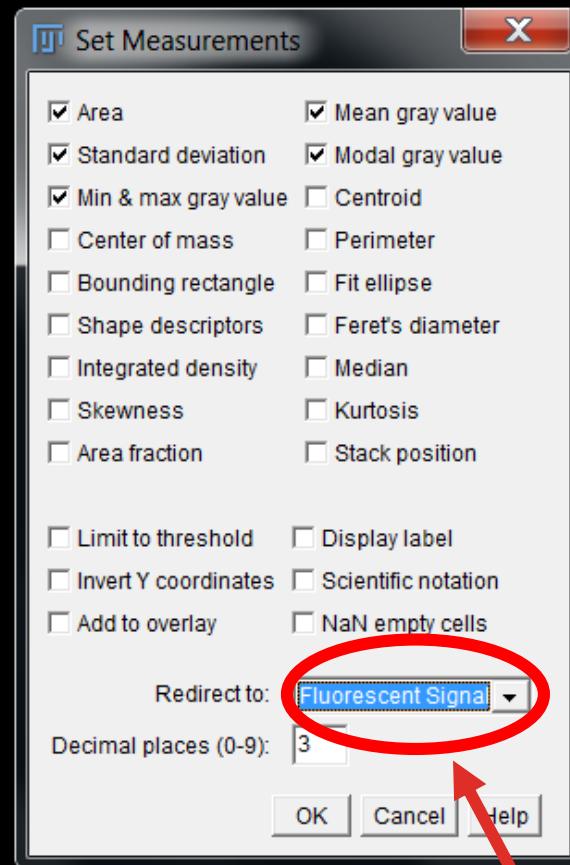
- 1. Using Particle Analyser to generate an ROI**
- 2. Apply ROI to image**
- 3. Measure the pixel values in the image within the ROI**



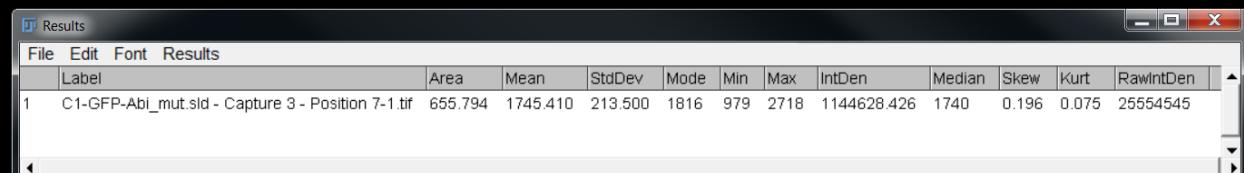
Binary Mask



Fluorescent Signal



FIJI will always apply measurements to whatever image is specified here

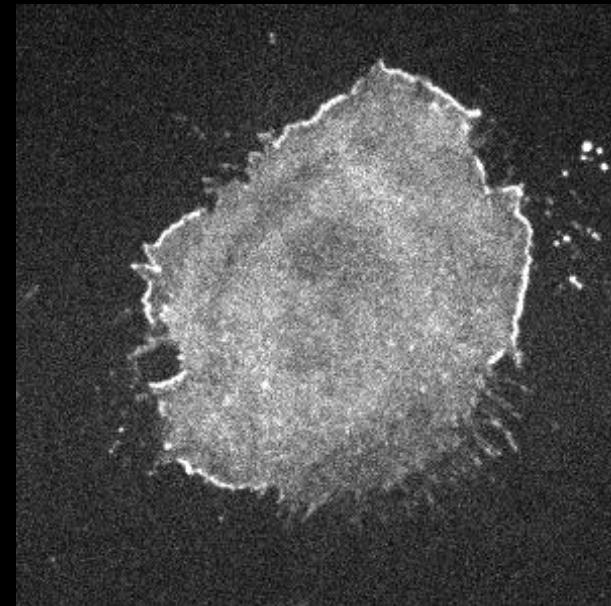
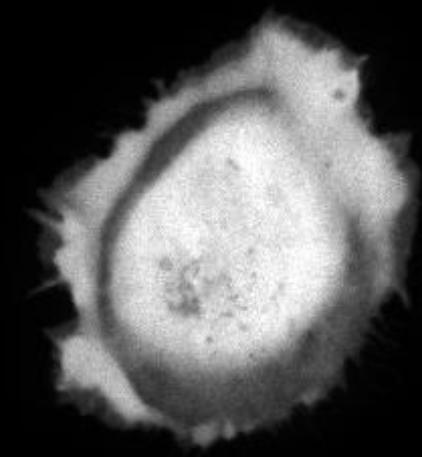
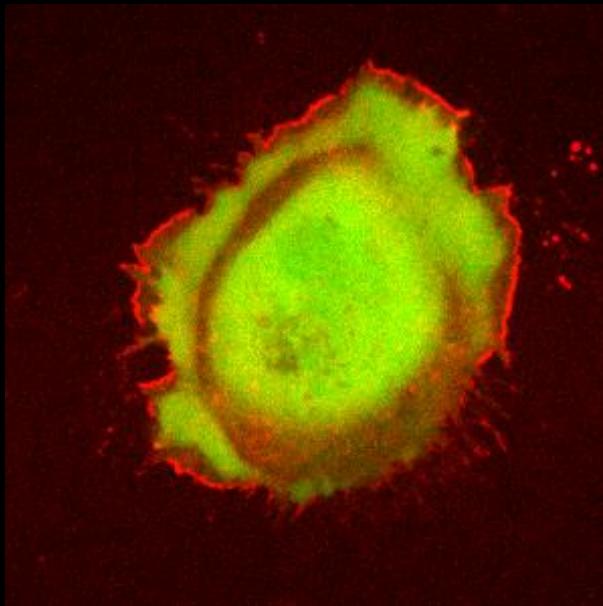


A screenshot of the 'Results' table window in FIJI. The table lists a single measurement for the file 'C1-GFP-Abi_mut.sld - Capture 3 - Position 7-1.tif'. The data includes:

Label	Area	Mean	StdDev	Mode	Min	Max	IntDen	Median	Skew	Kurt	RawIntDen
1 C1-GFP-Abi_mut.sld - Capture 3 - Position 7-1.tif	655.794	1745.410	213.500	1816	979	2718	1144628.426	1740	0.196	0.075	25554545

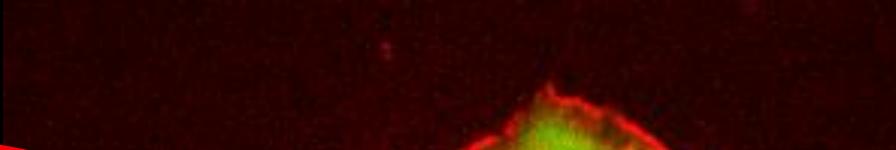
DEMO 8 – QUANTIFYING FLUORESCENCE

Demonstration of Particle Analyser's ability to apply ROIs to measure grey levels



WHAT IF WE WANT TO EXAMINE DIFFERENCES IN PROTEIN EXPRESSION...

...between here



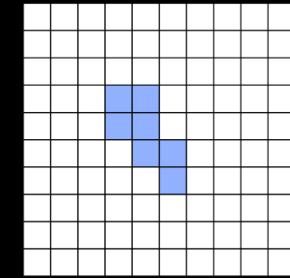
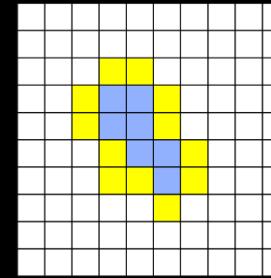
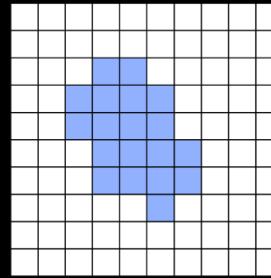
Require two different ROIs

- 1. Generate an ROI
- 2. Duplicate ROI and manipulate in some way
- 3. Apply ROIs to image, one at a time
- 4. Measure the pixel values in the image within the ROIs

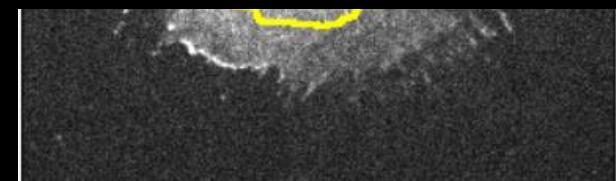


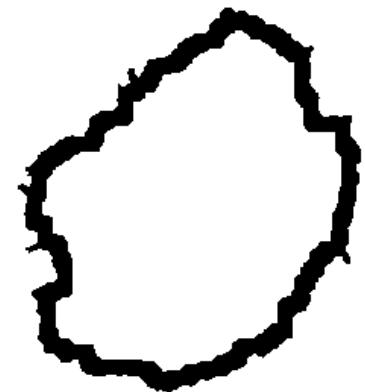
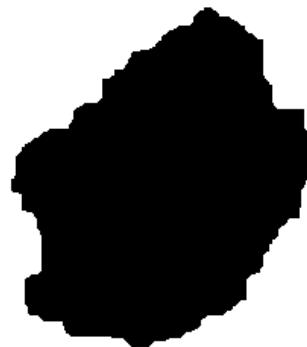
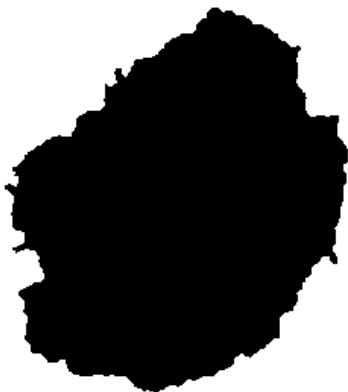
POSSIBLE TO MANIPULATE MASK PRIOR TO ROI GENERATION

Erosion



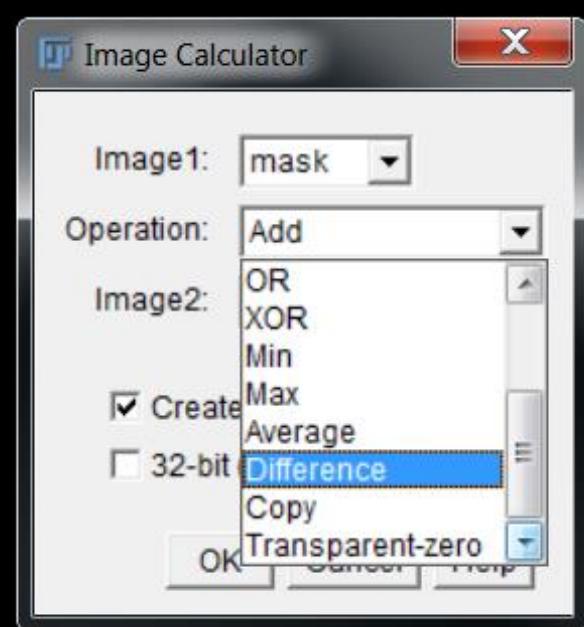
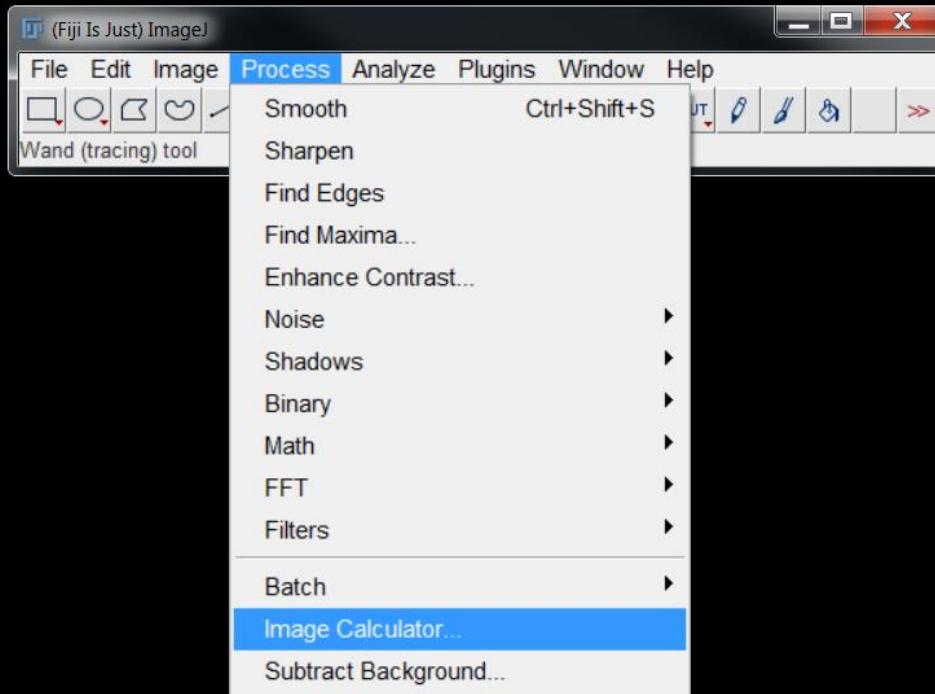
How do we generate a mask to represent the cell boundary?





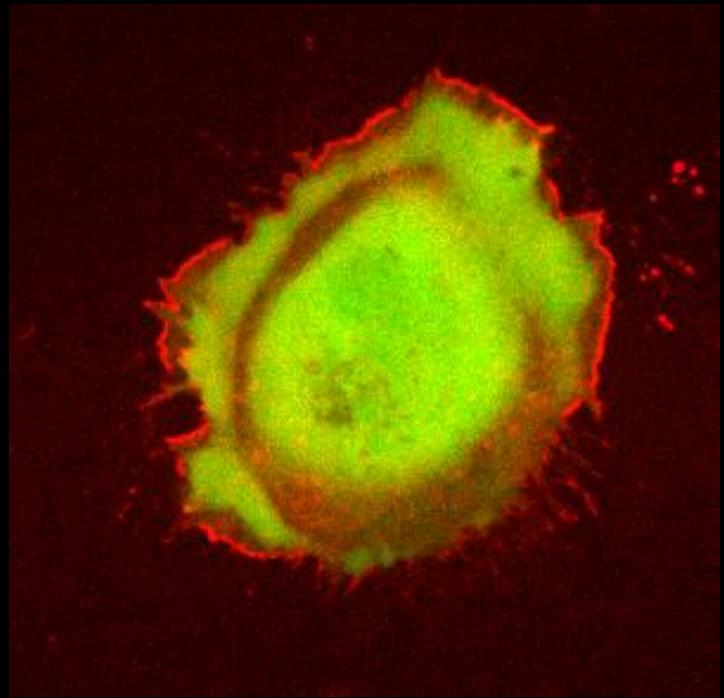
Calculate difference between these...

...to produce this.



CHALLENGE 2

Estimate the difference in mCherry expression between the cell edge and the cell centre



Filter Noise

- Process > Filters

Segment cells from background

- Image > Adjust > Threshold

Manipulate Segmentation

- Process > Binary

Create New Mask Image

- Process > Image Calculator

Specify Morphological Measurements

- Analyze > Set Measurements

Quantify Morphology & Fluorescence

- Analyze > Analyze Particles

CHALLENGE 2

Filter Noise

- Process > Filters

Segment cells from background

- Image > Adjust > Threshold

Manipulate Segmentation

- Process > Binary

Create New Mask Image

- Process > Image Calculator

Specify Morphological Measurements

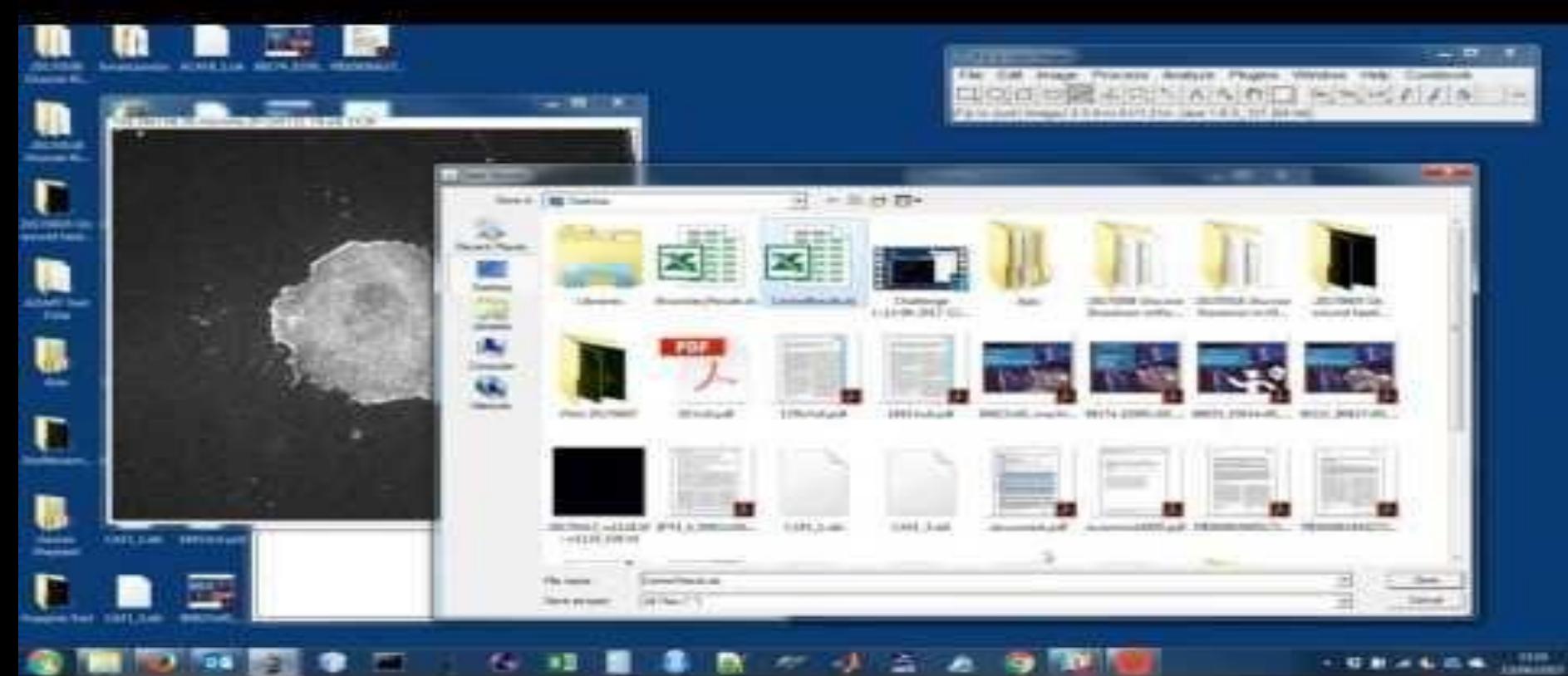
- Analyze > Set Measurements

Quantify Morphology & Fluorescence

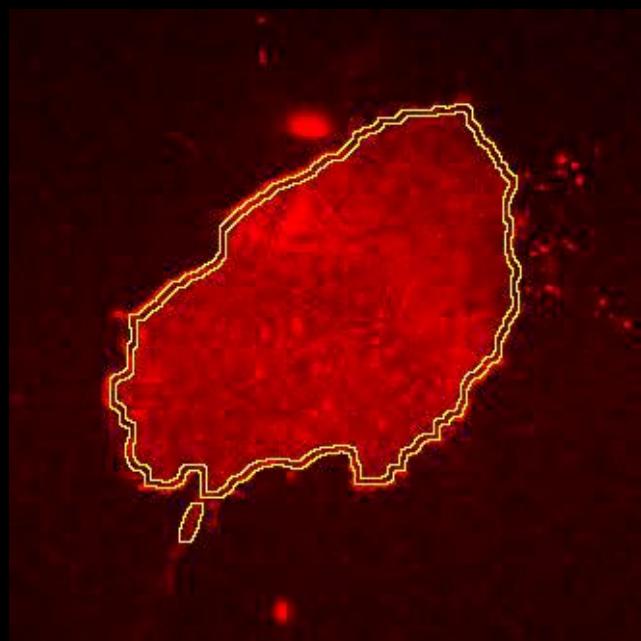
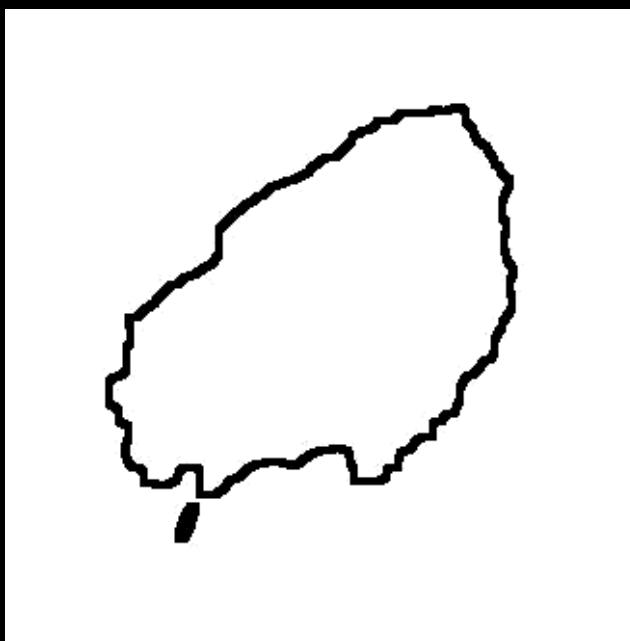
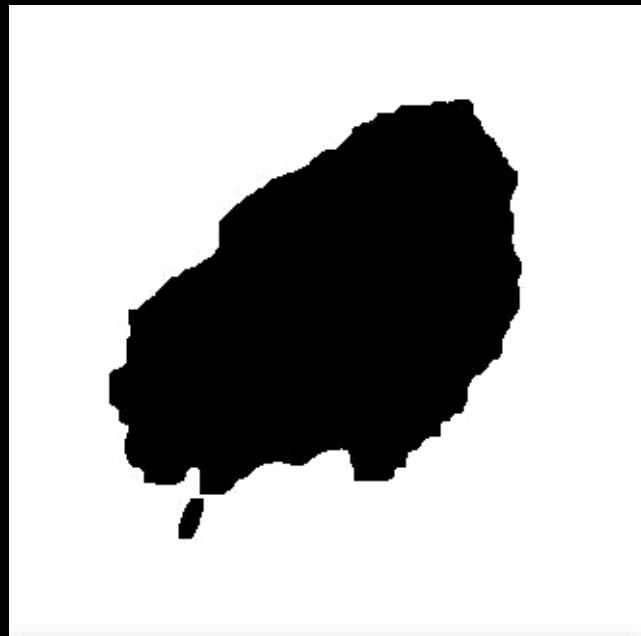
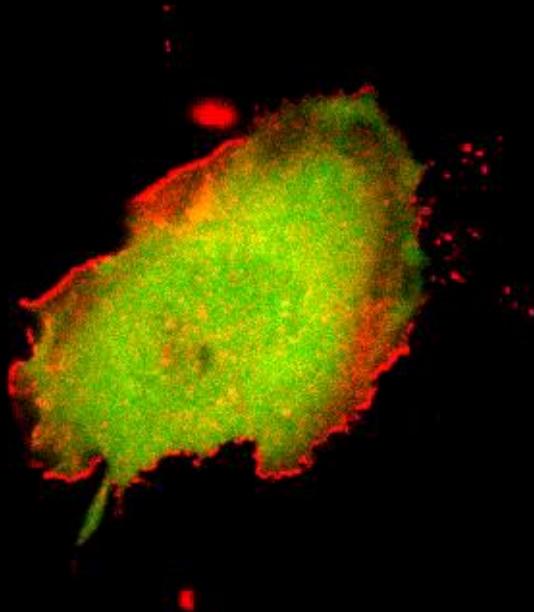
- Analyze > Analyze Particles

- Which variables in your analysis pipeline will have the greatest impact on your result?
- Is the result what you expected?
 - Why?
 - What could explain any discrepancy between what you see “visually” and what you obtain quantitatively.

SOLUTION TO CHALLENGE 2

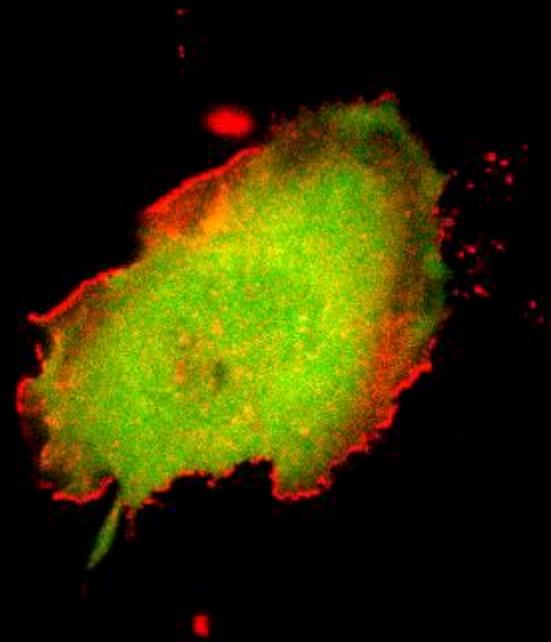


Everything we have done can also be applied to 3- & 4-D datasets



CHALLENGE 3

Determine whether mCherry localisation varies over time



Filter Noise

- Process > Filters

Segment cells from background

- Image > Adjust > Threshold

Manipulate Segmentation

- Process > Binary

Create New Mask Image

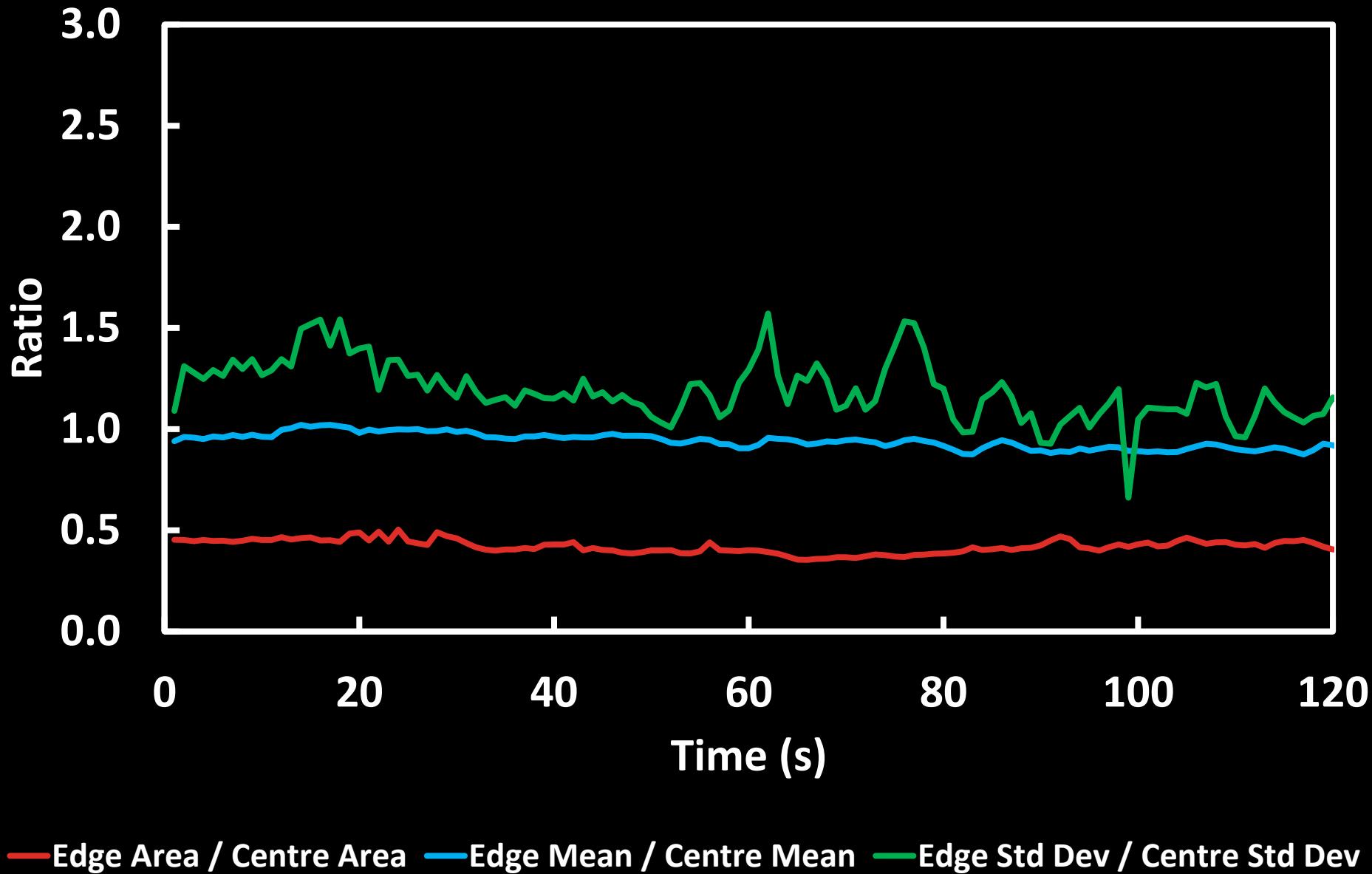
- Process > Image Calculator

Specify Morphological Measurements

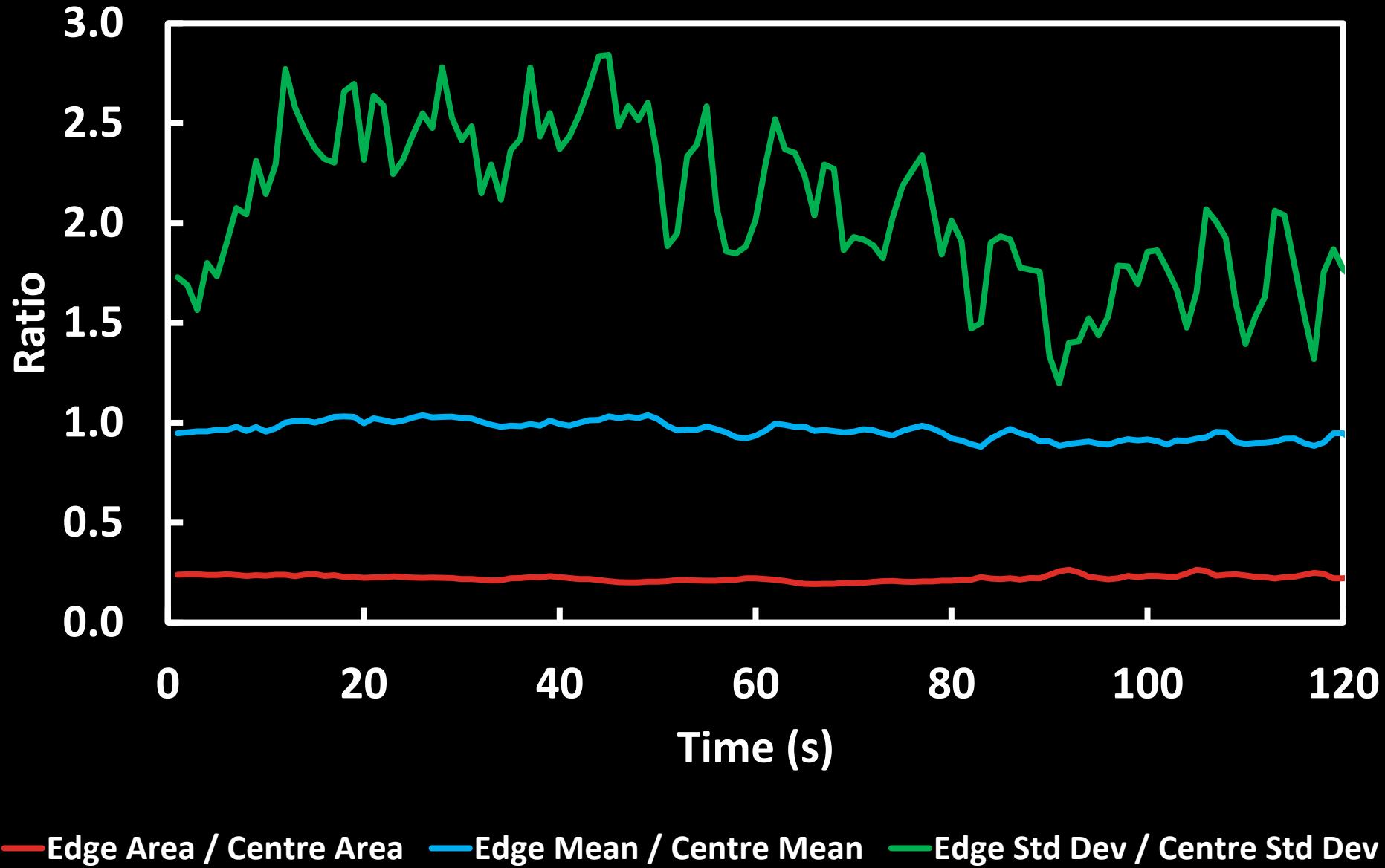
- Analyze > Set Measurements

Quantify Morphology & Fluorescence

- Analyze > Analyze Particles

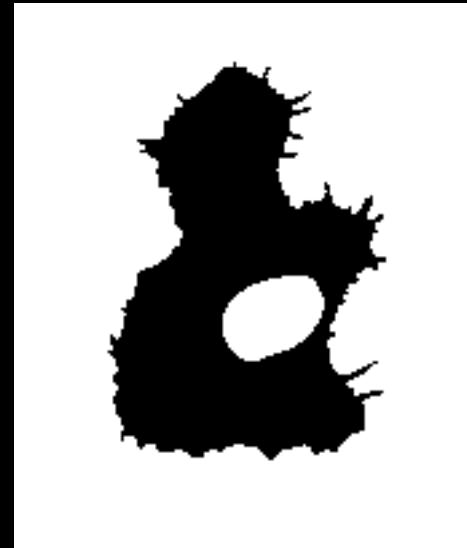
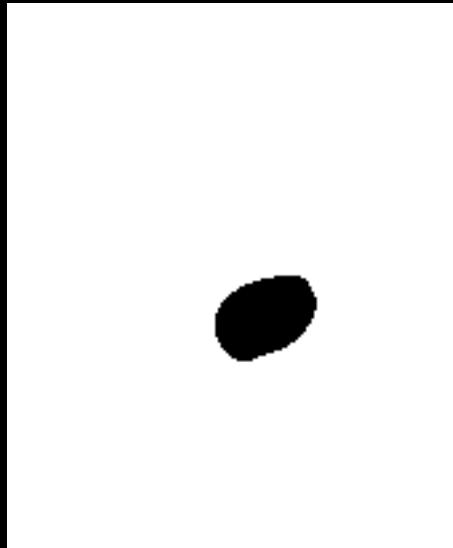
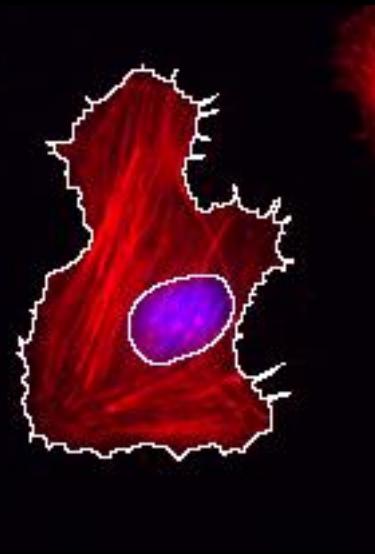
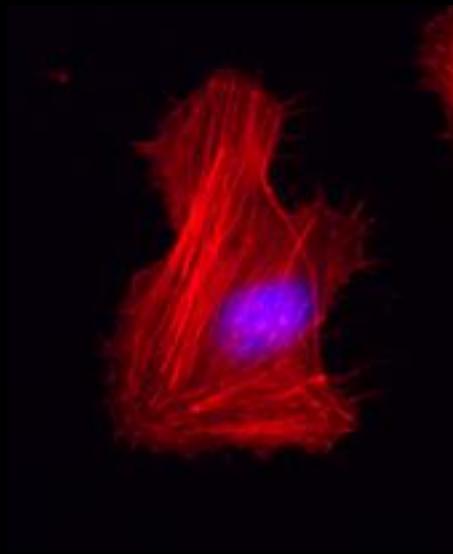
$N_{erosions} = 4$ 

$$N_{erosions} = 2$$



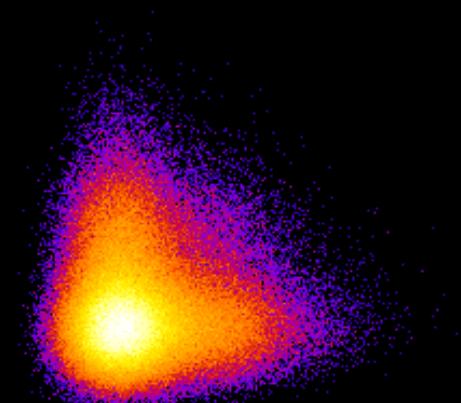
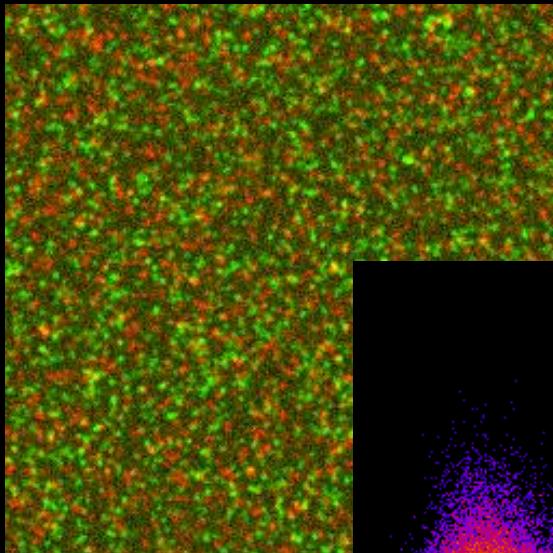
EASY TO EXTEND THIS APPROACH...

For example, to compare nuclear & non-nuclear expression



ADVANCED TOPICS

- Object Tracking
- Colocalisation analysis
- Writing macros & plugins



```
9 import ij.plugin.frame.RoiManager as RM
10 import fiji.plugin.kymographbuilder.KymographFactory as KFactory
11
12
13 def validDataset(dataset):
14     """Assess if dataset has suitable dimensions"""
15     from net.imagej.axis import Axes
16     z = dataset.dimension(dataset.dimensionIndex(Axes.Z))
17     t = dataset.dimension(dataset.dimensionIndex(Axes.TIME))
18     return z * t > 1
19
20
21 rm = RM.getInstance()
22 counter = 0
23 if validDataset(dataset) and rm and rm.getCount():
24     for roi in rm.getRoisAsArray():
25         if roi.isline():
26             kfactory = KFactory(context, dataset, roi)
27             kfactory.build()
28             counter += 1
29             title = "Kymograph" + str(counter).zfill(3) + "_" + roi.getName()
30             ij.ui().show(title, kfactory.getKymograph())
31             log.info("MultiKymographBuilder Finished. " + str(counter) + " ROIs processed")
32 else:
33     log.error("Either the ROI Manager is empty or " + dataset.getName() + " has invalid dimensions")
```

Bioinformatics Training

You are not currently logged in [Log in](#)[Home](#)[Your bookings](#)[Timetable](#)[Courses](#)[Themes](#)[Venues](#)**Mon 26 Jun - Wed 28 Jun 2017**

09:30, ...

Venue: Bioinformatics Training Room, Craik-Marshall Building, Downing Site

Provided by: Bioinformatics

Booking

Bookings cannot be made on this event (Event is completed).

Other dates:

No more events

[\[Show past events \]](#)**Register interest**

Register your interest - if you would be interested in additional dates being scheduled.

Image Analysis for Biologists

 Map**Description**

This course will focus on **computational methods for analysing cellular images** and **extracting quantitative data from them**. The aim of this course is to familiarise the participants with computational image analysis methodologies, and to provide hands-on training in running quantitative analysis pipelines.

On day 1 we will introduce **principles of image processing and analysis**, giving an **overview of commonly used algorithms** through a series of talks and practicals based on **Fiji**, an extensible open source software package.

On day 2, we will describe the open **Icy platform** developed at the Institut Pasteur. Icy is a next-generation, user-friendly software offering powerful **acquisition, visualisation, annotation and analysis algorithms for 5D bioimaging data**, together with unique automation/scripting capabilities (notably via its graphical programming interface) and tight integration with existing software (e.g. ImageJ, Matlab, Micro-Manager).

On day 3, we will cover time series processing and cell tracking using **TrackMate**. In the afternoon, we will present the **Image Data Resource**, an added-value platform that combines data from multiple independent imaging experiments and imaging modalities and integrates them into a single resource for reanalysis in a convenient, scalable form.

Please note that if you are not eligible for a University of Cambridge Raven account you will need to book or register your interest by linking [here](#).

Target audience

- Researchers who are applying or planning to apply image analysis in their research
- **Graduate students, Postdocs and Staff members** from the **University of Cambridge**, **Affiliated Institutions** and other external Institutions or individuals
- Please be aware that these courses are **only free for University of Cambridge students**. All other participants will be charged a registration fee in some form. Registration fees and further details regarding the charging policy are available [here](#)
- Further details regarding eligibility criteria are available [here](#)

Prerequisites

Basic skills in mathematics and programming are an advantage, but not a requirement.

Sessions

Number of sessions: 3

#	Date	Time	Venue	Trainers
1	Mon 26 Jun 2017	09:30 - 17:30	Bioinformatics Training Room, Craik-Marshall Building, Downing Site	 Richard Butler, Jeremy Pike

ONLINE COURSE

Image Analysis Methods for Biologists

Get an introduction to image acquisition and analysis for biologists – from basic techniques to the future of image analysis.

[Join course](#)[Overview](#)[Start dates](#)[Requirements](#)[Educators](#)

Duration
4 weeks



3 hours
per week



FREE
online course



Upgrade
available

[What's this?](#)

[Support](#)

<https://www.futurelearn.com/courses/image-analysis>

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Pin this

Image analysis help desk

Email alerts

Contribute

Sharing knowledge and expertise to help research analyse image datasets.

Meets 2nd and 4th Wednesday of the month at
16:00 in the Crick bar.

Many of you are involved in acquiring microscopy image datasets and one of the aims of the Light Microscopy STP is to equip you with the necessary knowledge and expertise to analyse such datasets effectively.

However, we are aware that this may seem daunting to the uninitiated.

With this in mind, we have created an informal **Image Analysis "Hacking Club"**.

This will involve us situating ourselves in the coffee bar area from 16:00 (we will attempt to make ourselves easily identifiable) and anyone can drop by to ask us any kind of image-analysis related questions you can think of. This may be, for example, some pointers on analysing some images you have already acquired, or advice on designing future

IMAGEJ



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Fiji

Fiji is an image processing package—a "batteries-included" distribution of [ImageJ](#), bundling a lot of plugins which facilitate scientific image analysis.

- **For users** - Fiji is [easy to install](#) and has an automatic update function, bundles a [lot of plugins](#) and offers comprehensive documentation.
- **For developers** - Fiji is an open source project hosted in a [Git](#) version control [repository](#), with access to the source code of all internals, libraries and plugins, and eases the [development](#) and [scripting](#) of plugins.

[Contents \[hide\]](#)

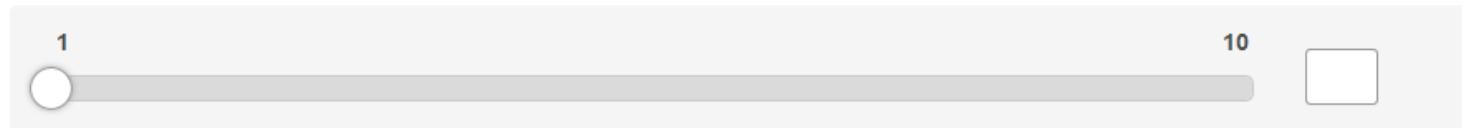
1 Downloads
2 License
3 Contributing
4 Publication

 **Fiji Is Just ImageJ**

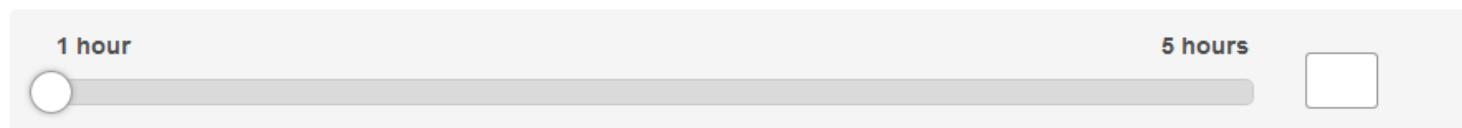
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[Using Fiji](#)
[Featured Fiji Projects](#)
[Fiji Publications](#)
[Links](#)

[Developing Fiji \[-\]](#)
[Building Fiji from source](#)
[Developing Fiji](#)

* 1. On a scale of 1 to 10, where 10 represents the highest level, what was your overall level of satisfaction with the workshop?



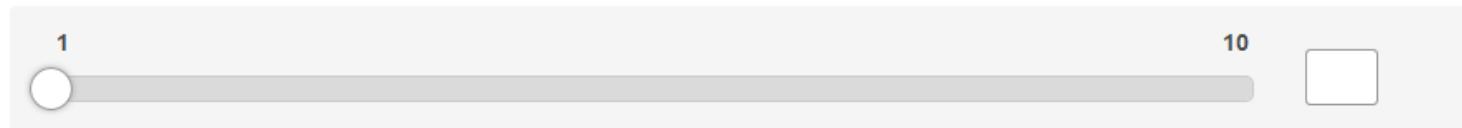
* 2. The workshop ran for approximately three hours - what do you think the ideal length would be?



3. How did you find the balance between lecturing and practical demonstrations?

- Too much time was spent explaining theory and providing background information.
- The balance was about right
- Not enough time was spent explaining theory and providing background information.

* 4. On a scale of 1 to 10, where 10 represents the highest level, how difficult did you find the challenges?



Have a Nice Weekend

