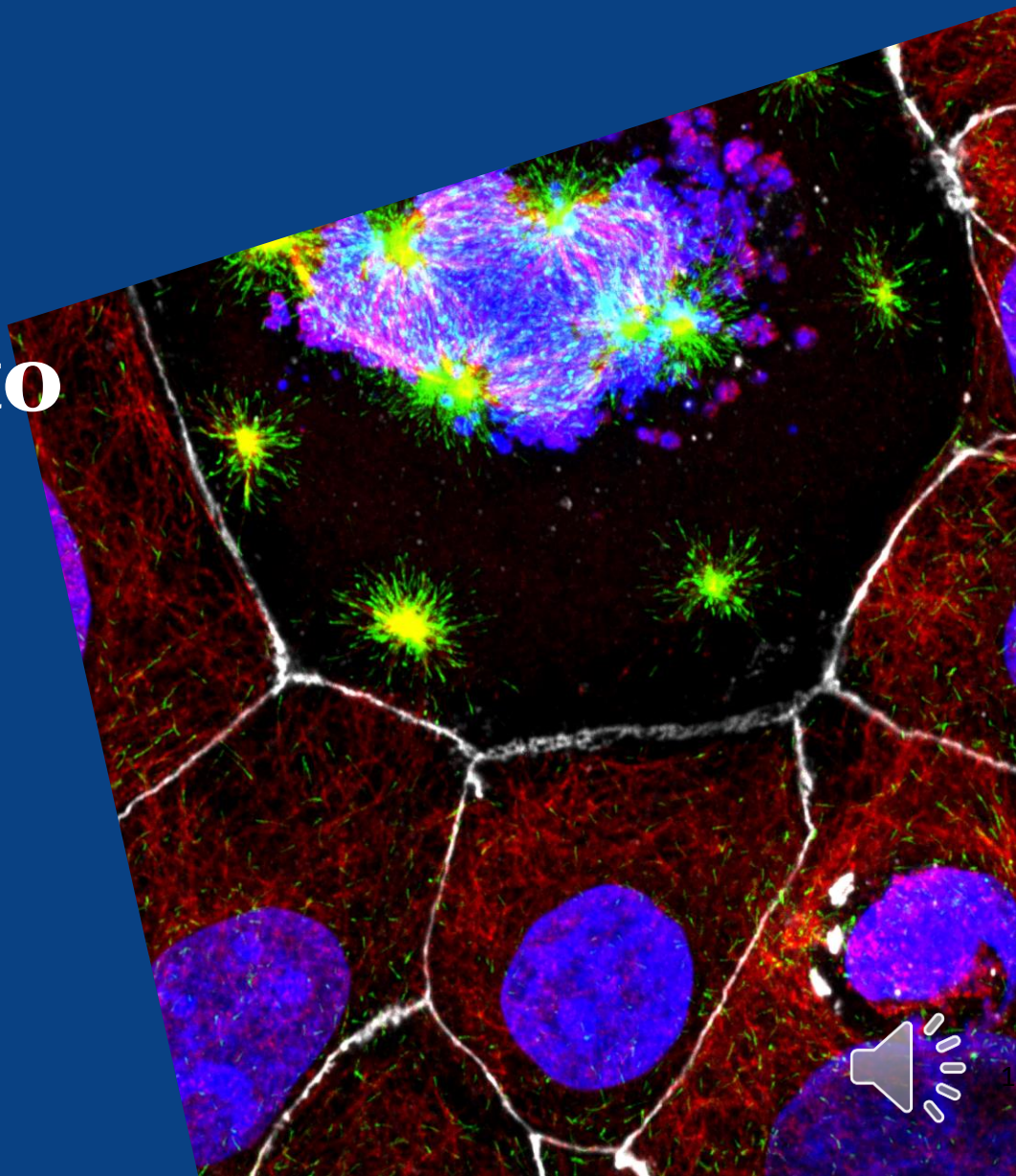
THE UNIVERSITY OF MELBOURNE

Introduction to Bioimage Analysis



Dr Ellie Cho & Dr Shane Cheung

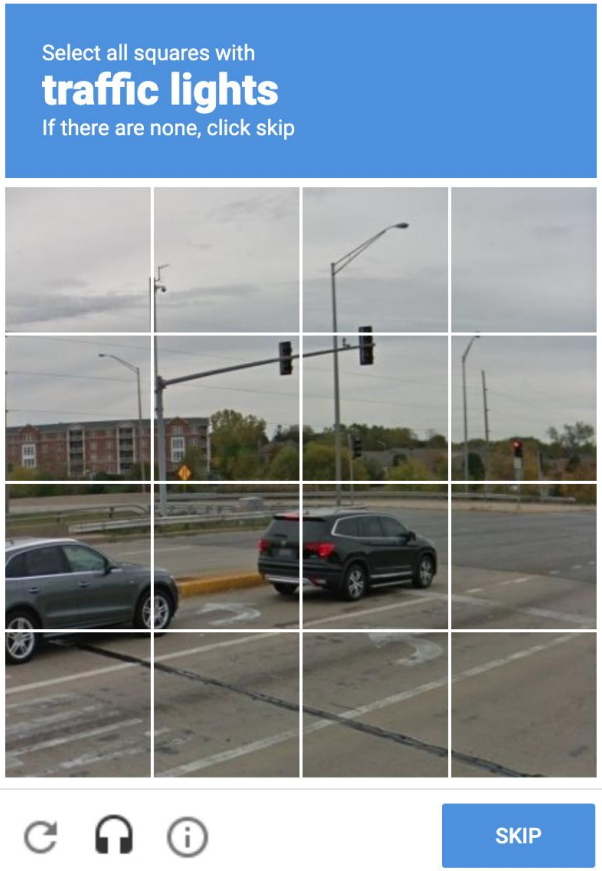
Platform Manager /Application Specialists

Biological Optical Microscopy Platform

Part 1.
An Overview of Bioimage Analysis

What is an object?

What is trivial for a human can be very difficult for a computer:

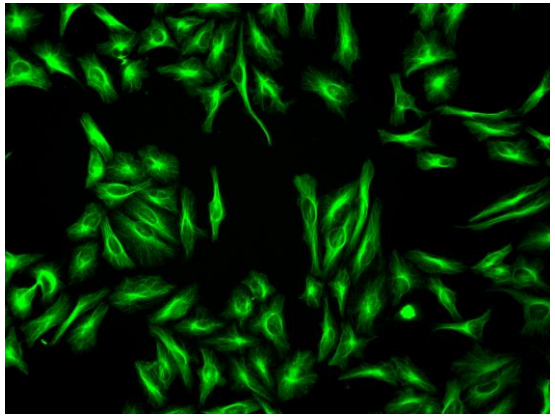


Bioimage analysis

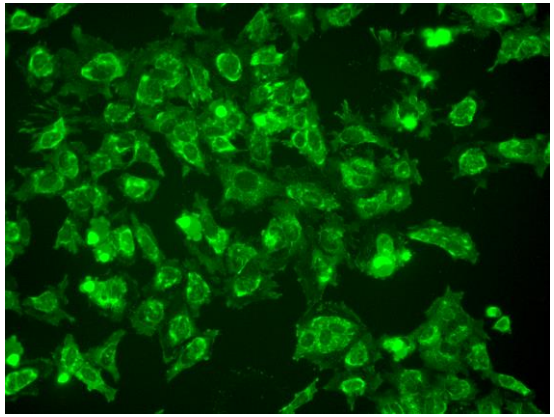
Goal::

Obtaining quantifiable information from microscopy image of biological sample

Control



Treated



In treated group, cell looks ‘rounder“…

But how much?

Bioimage analysis

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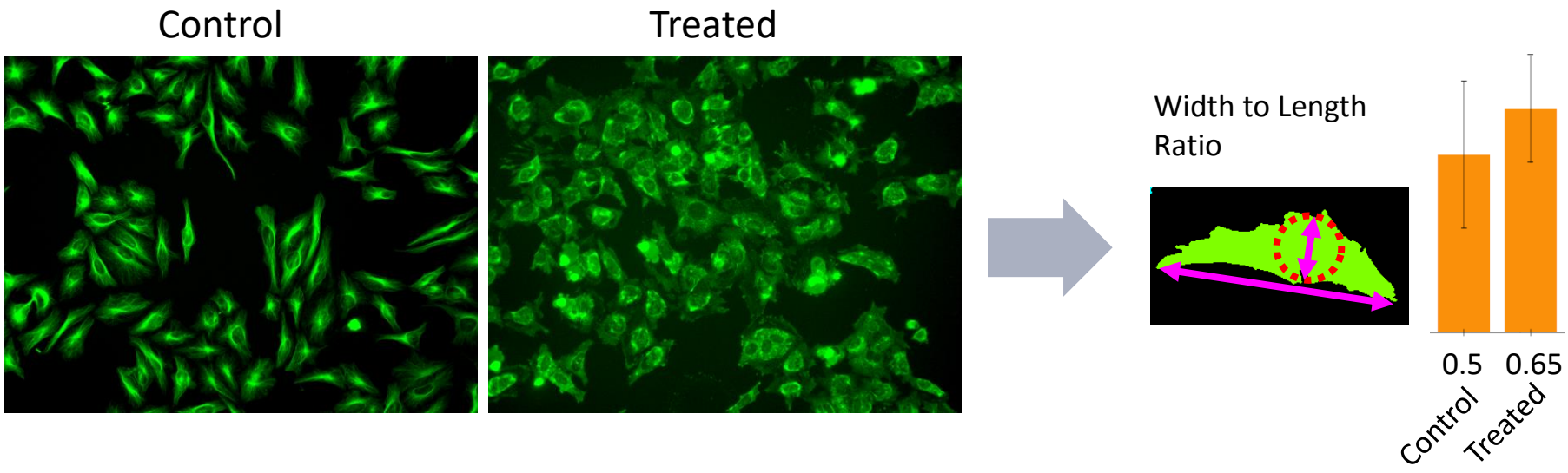
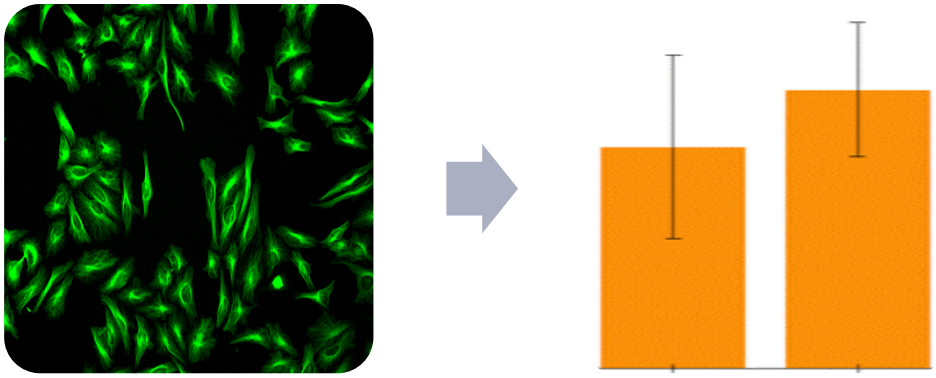
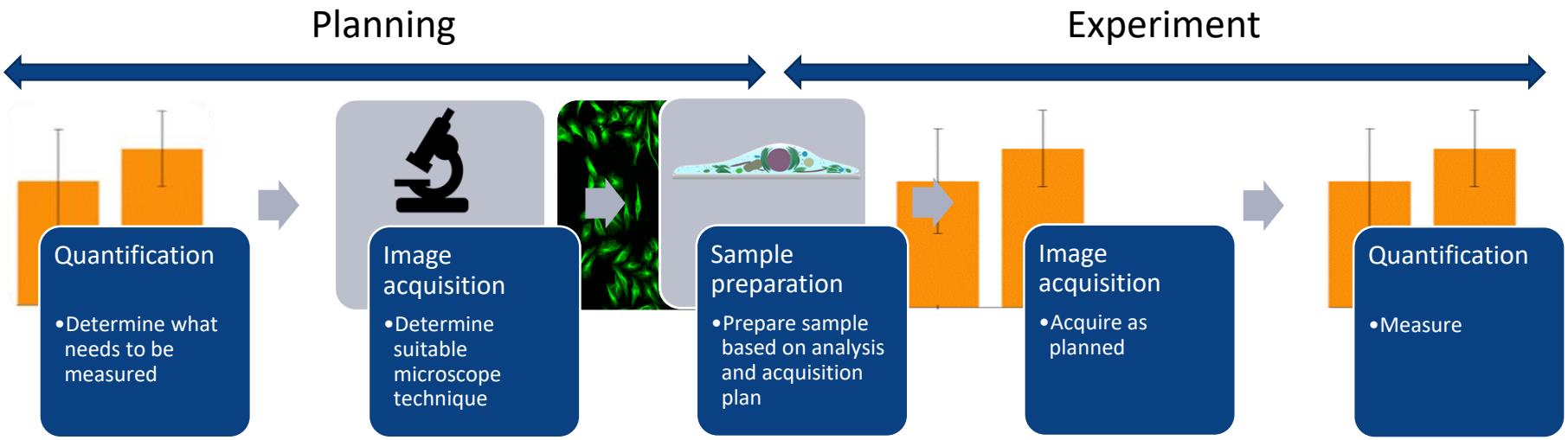


Planning for bioimage analysis



Planning for bioimage analysis



Planning for bioimage analysis

Planning

Experiment

Quantification
- Determine what needs to be measured

Image acquisition
- Determine suitable microscope technique

Sample preparation
- Prepare sample based on analysis and acquisition plan

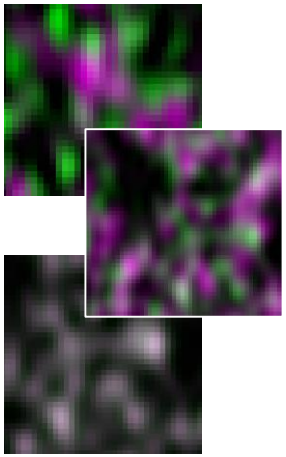
Image acquisition
- Acquire as planned

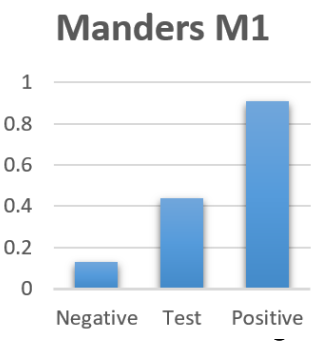
Quantification
- Measure

Colocalisation of 2 proteins in mitochondria

Super-resolution to resolve inner & outer membrane of mitochondria

Monolayer cell culture on coverslip
- Test sample
- Negative control
- Positive control





Planning for bioimage analysis

Planning

Experiment

Quantification
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Image acquisition
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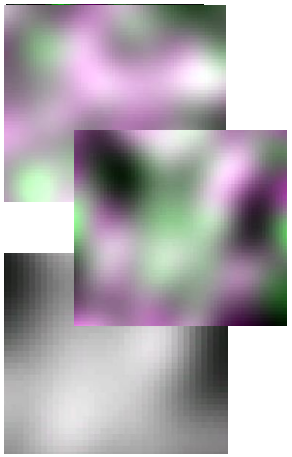
Quantification
- Measure

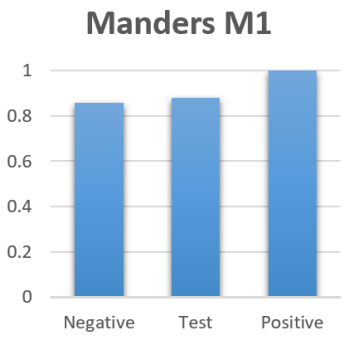
Colocalisation of 2 proteins in mitochondria

Super-resolu… to resolve in… outer … brane of mit… ndria

Low-resolution?

Monolayer cell culture on coverslip
- Test sample
- Negative control
- Positive control





Planning for bioimage analysis

Planning

Experiment

Quantification
- Determine what needs to be measured

Image acquisition
- Determine suitable microscope technique

Sample preparation
- Prepare sample based on analysis and acquisition plan

Image acquisition
- Acquire as planned

Quantification
- Measure

Run pilot

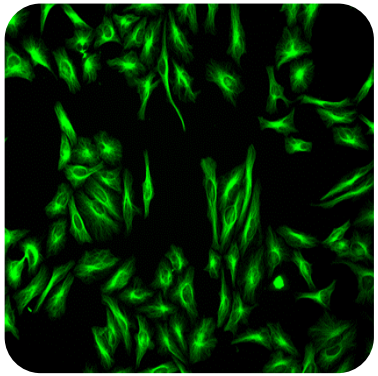
Run Full scale

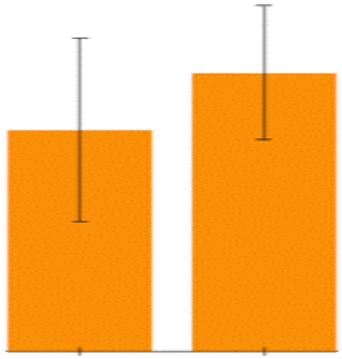
Consult with us

Biological Optical Microscopy Platform

bomp-enquiries@unimelb.edu.au

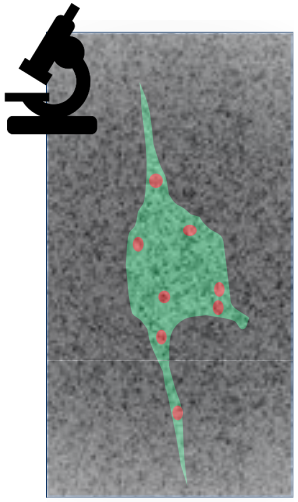
Image Analysis workflows





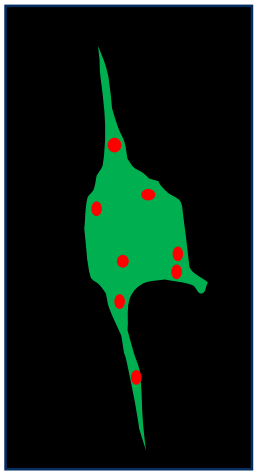
General workflows

Acquisition

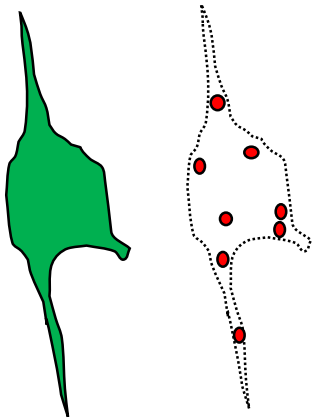


Raw microscopy image

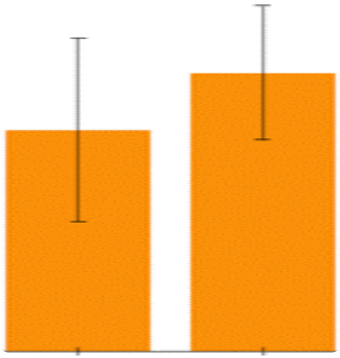
Pre-processing



Object Segmentation



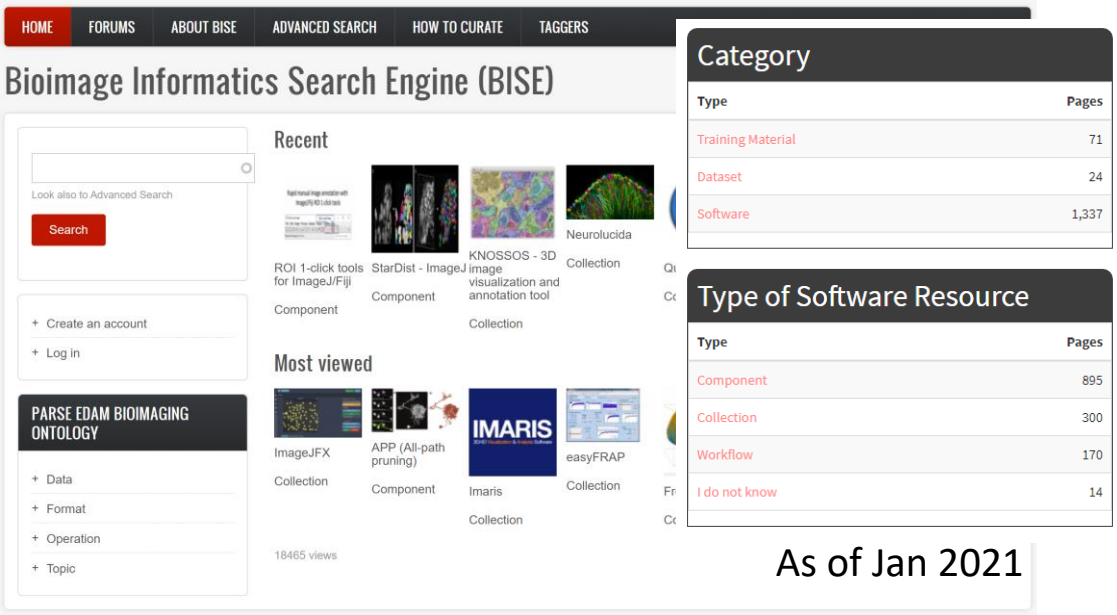
Measurement / Data analysis



ZEN + + + Imaris BITPLANE

Image processing software

https://biii.eu/
by Networks of European Bio-image analyst (NEUBIAS)



As of Jan 2021

Reviews
Eliceiri et al., Nat Methods, 2012
Wiesmann et al., J Microsc, 2015
Baroux et al., Methods Mol Biol, 2018

Software name	Primary function
ImageJ	Image analysis
Fiji	Image analysis
BioImageXD	Image analysis
Icy	Image analysis
CellProfiler	Image analysis
Vaa3D	Visualization and image analysis
FarSight	Visualization
VTK	Bioimaging library
ITK	Bioimaging library
OpenCV	Bioimaging library
WND-CHARM	Machine learning
PSLID	Machine learning
Ilastik	Machine learning
CellProfiler Analyst	Machine learning and data analysis
PatternUnmixer	Machine learning
CellOrganizer	Machine learning, modeling and visualization
KNIME	Workflow system

(Eliceiri 2012)

Image processing software @ BOMP

Freeware

ImageJ/Fiji

CellProfiler™
cell image analysis software

ilastik

Commercial

ZEN ZEISS

LEICA

NIS Elements
NIKON

FV Olympus

Harmony®
Perkin Elmer

SVI

Volocity

MetaMorph®

Amira®

BOMP Workshop Series

Upcoming image analysis workshops from BOMP:

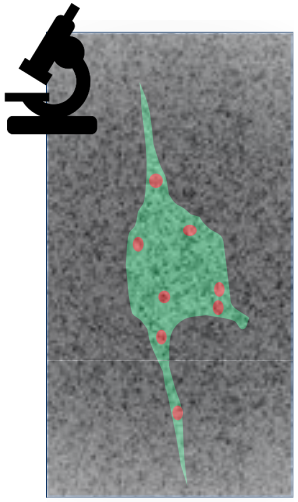
- FIJI/Image J for Beginners
- FIJI/Image J for Quantification
- Introduction to: CellProfiler
- FIJI/Image J FIJI Macro writing for biologists
- Colocalisation
- 3D image analysis (Imaris)
- Filaments
- Tracking
- Deconvolution (Huygens)

https://microscopy.unimelb.edu.au/

https://microscopy.unimelb.edu.au/news-and-events/bomp-workshop-in-2021

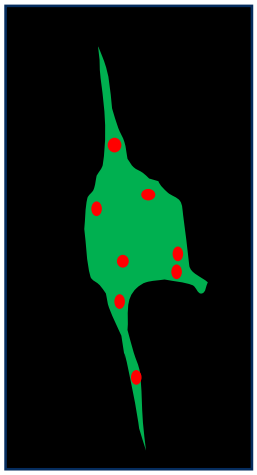
Part 2.
The Bioimage Analysis Workflow

Acquisition

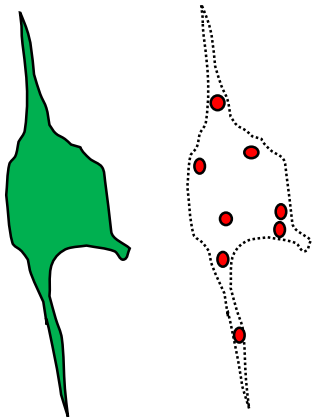


RAW Image

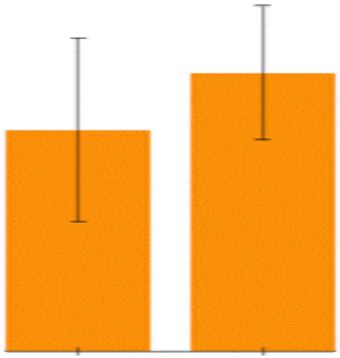
Pre-processing



Object Segmentation

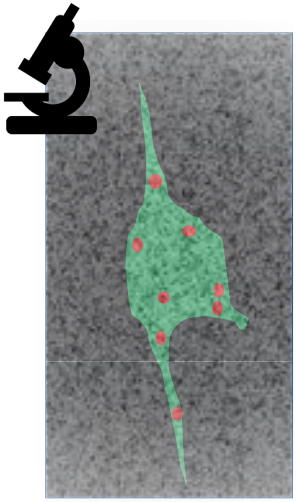


Measurement / Data analysis



Acquisition

Pre-processing Object detection Measurement / Data analysis

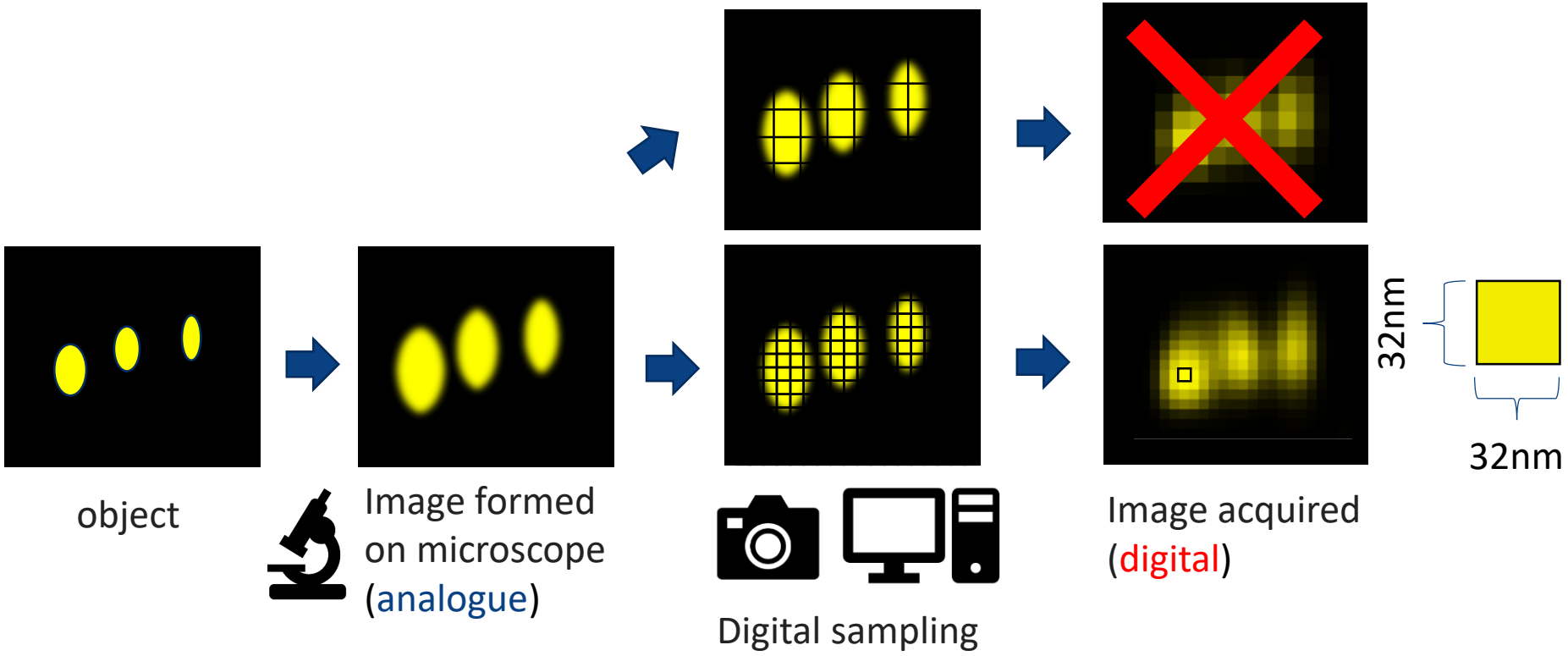


Raw microscopy image

During acquisition

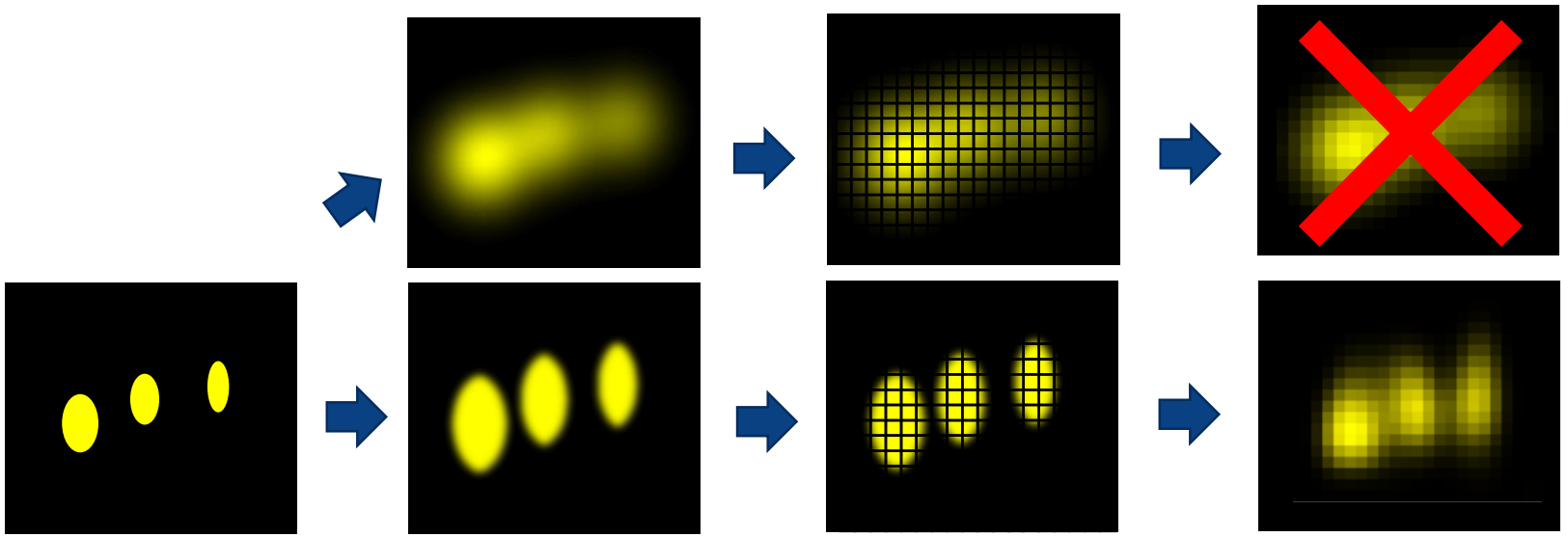
- Acquire with sufficient **sampling rate**
- Avoid **saturation**
- Use proper **bit depth**
- Use whole **dynamic range**
- Use appropriate **resolution**

What is Microscopy Image



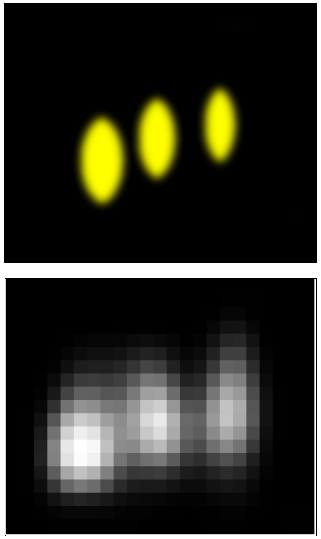
Acquire with sufficient **sampling rate**

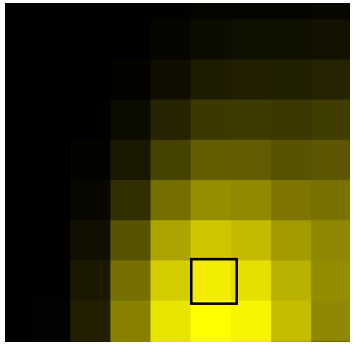
Raw Microscopy Image



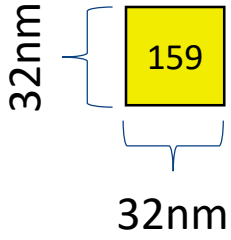
Appropriate **resolution** for our object

Raw Microscopy Image





0	0	0	1	4	8	11	11	12
0	0	0	2	10	19	21	21	25
0	0	1	7	24	39	40	38	42
0	0	2	16	45	64	64	57	59
0	0	5	32	76	97	94	81	79
0	1	11	57	112	135	128	105	93
0	1	17	76	139	159	151	121	96
0	1	21	88	154	170	165	128	93

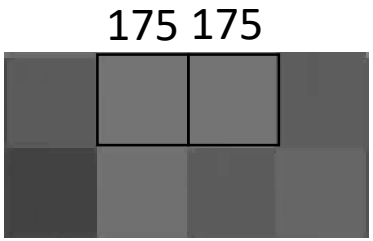


2^1

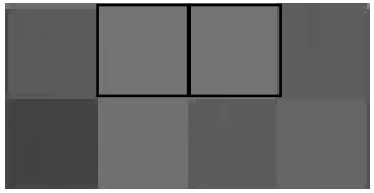
2^{12}

2^{16}

8bit

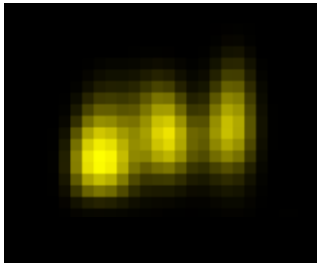


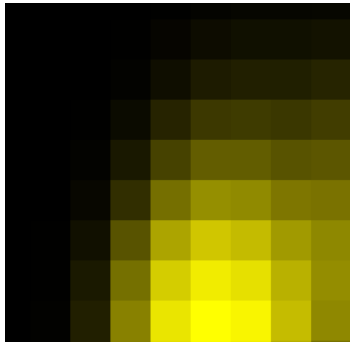
16bit



Use higher **Bit depth** for intensity measurement

Raw Microscopy Image





0	0	0	1	4	8	11	11	12
0	0	0	2	10	19	21	21	25
0	0	1	7	24	39	40	38	42
0	0	2	16	45	64	64	57	59
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0	1	11	57	112	135	128	105	93
0	1	17	76	139	159	151	121	96
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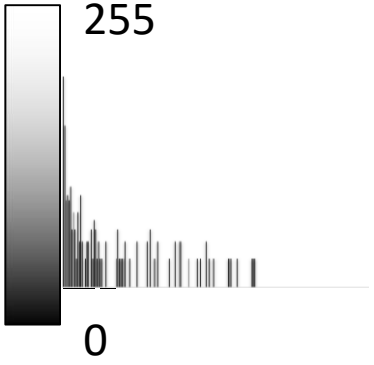
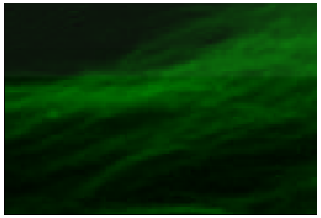
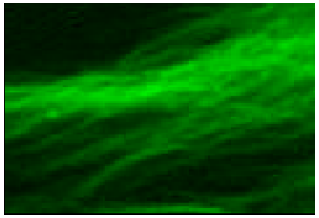
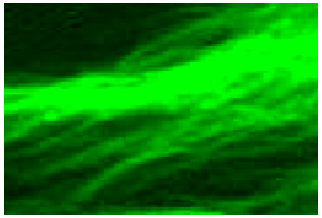
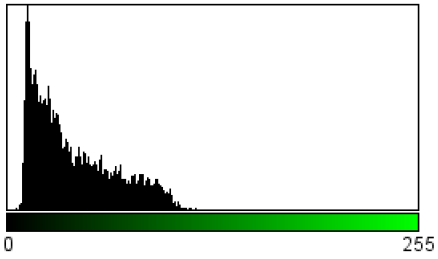


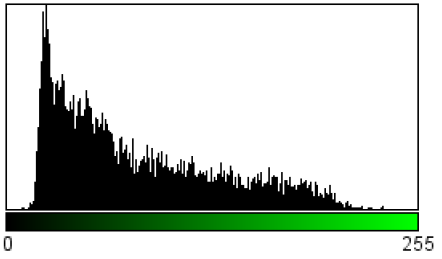
Frequency Histogram

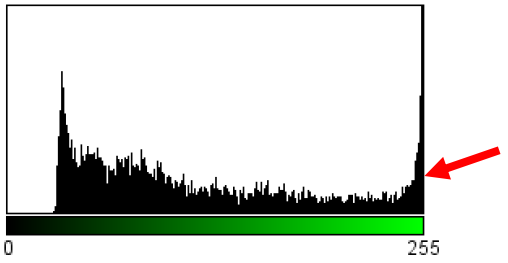












Use whole **dynamic range**

Avoid **saturation**

During acquisition

- Image to be compared **MUST** be acquired (and processed) using the same settings

- Save the image using the microscopy raw format (CZI, LIF, OIB, ND2… OME-TIFF) to preserve metadata. Avoid saving as standard TIFF, JPG, PNG…

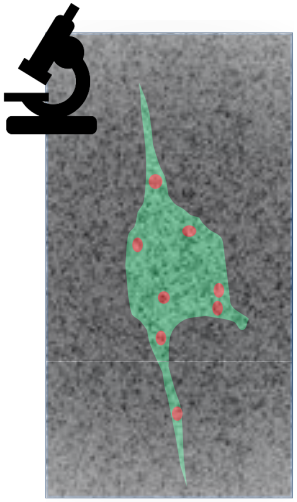
More detail on our other microscopy seminar series

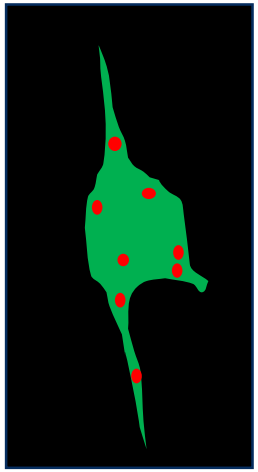
https://microscopy.unimelb.edu.au/optical-microscopy/workshops-resources

Pre-processing

Object detection

Measurement / Data analysis

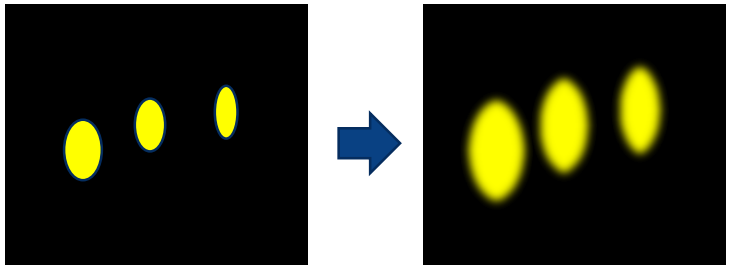




Raw microscopy image

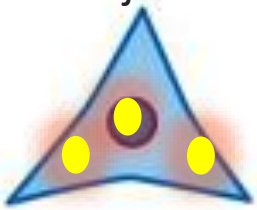
Pre-processing

- De-noising
- Background correction
- Bleaching correction (in time, in Z)
- Alignment
- Deconvolution



Fluroescent signal from object

Image formed on microscope

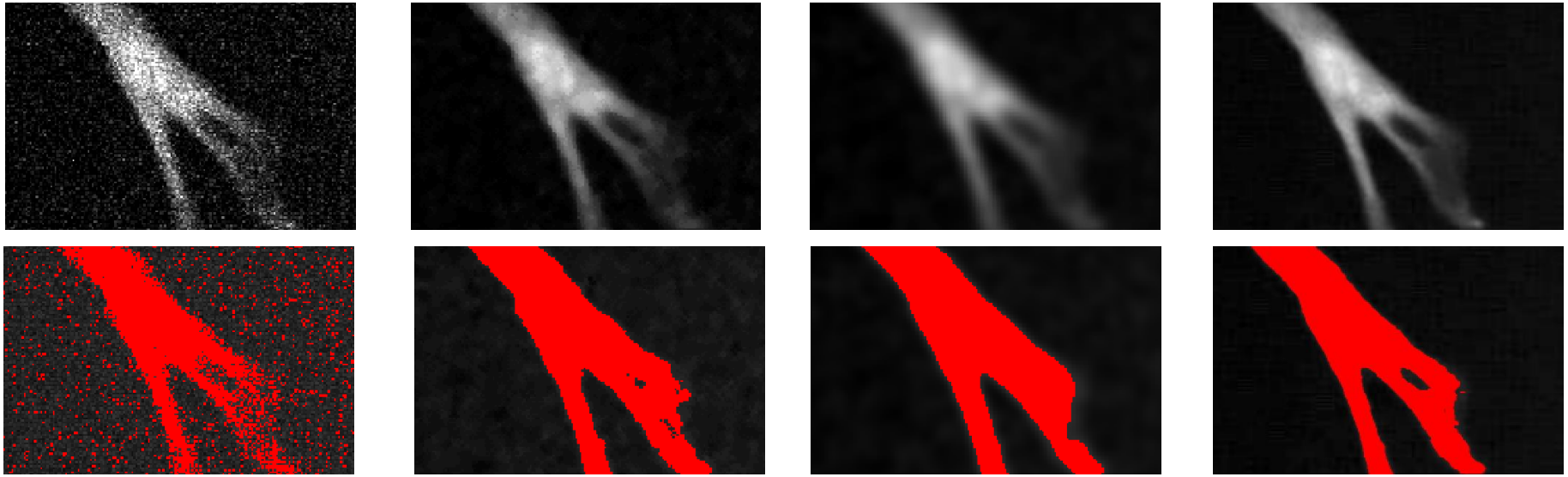


De-noising

Median Filter
(preserves edge)

Gaussian Filter
(smoothing,blurring)

Deep learning



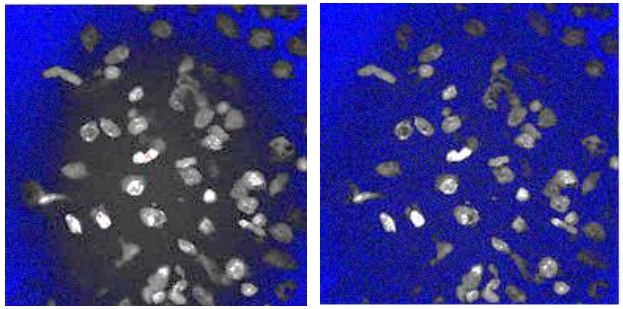
The Image Processing Handbook 7th
(Russ & Neal 2016)

Noise2Void (Krull 2019)
https://github.com/juglab/n2v

Image : Celltrackingchallenge dataset Fluo-C2DL-MSC

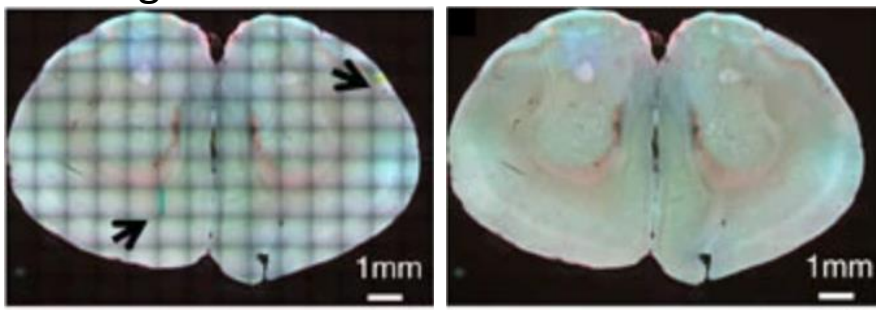
Background correction

Background subtraction



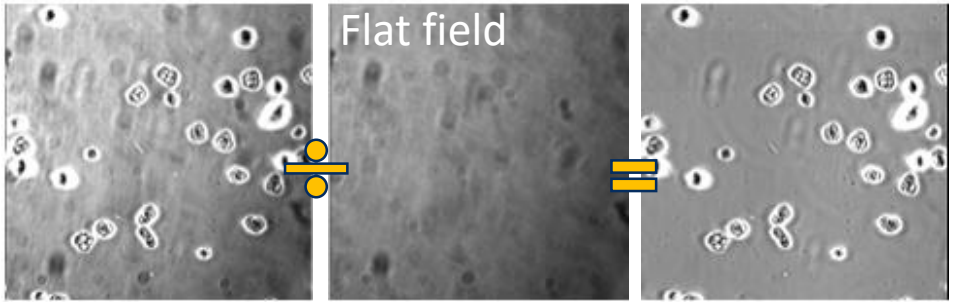
'Rolling Ball' (Castle and Keller 2007)

Shading correction

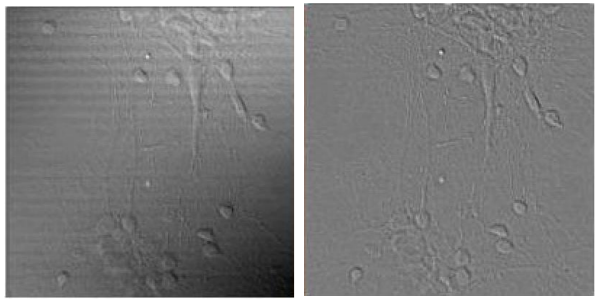


BaSiC (Peng 2017)

Flat Field Correction



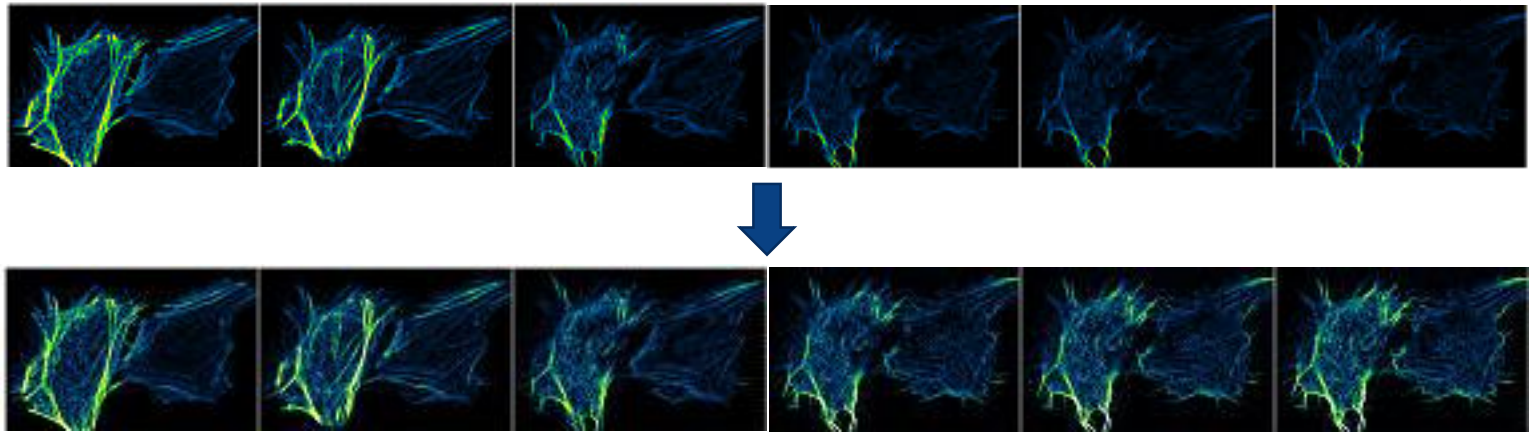
Suppress stripes (Bandpass Filter)

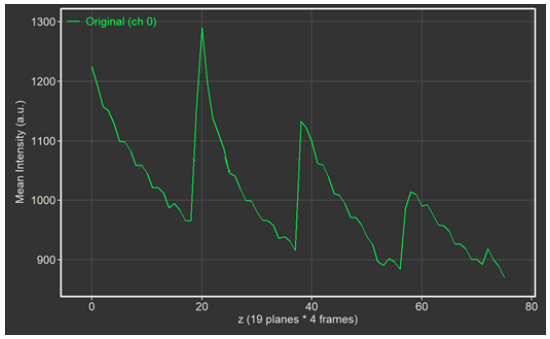


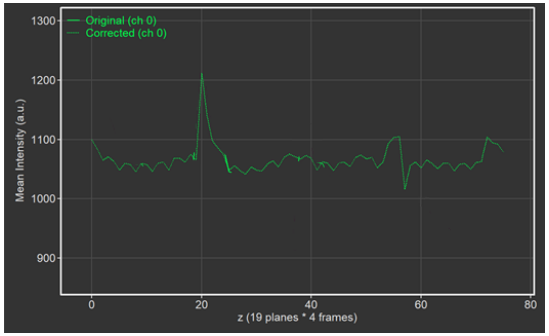
https://imagej.net/Image_Intensity_Processing#Background_correction

Bleaching correction

Over time or over Z stsack





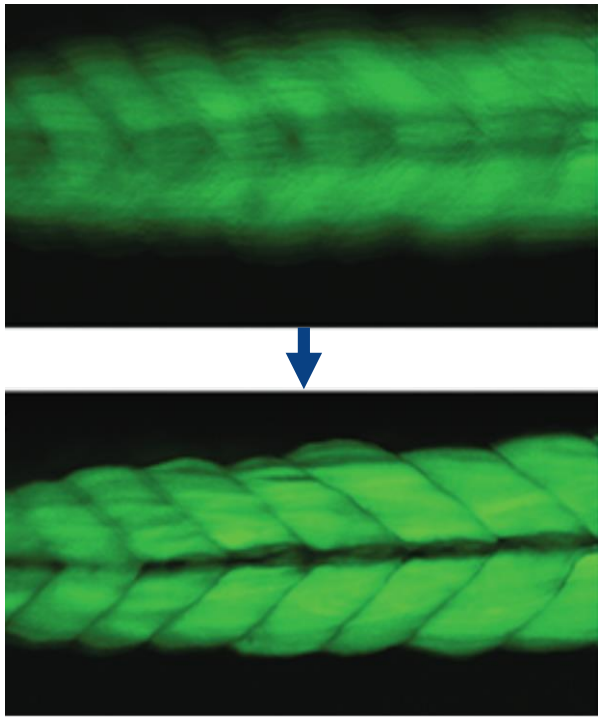


‘Bleach Correction’

‘Bleaching Corrector’

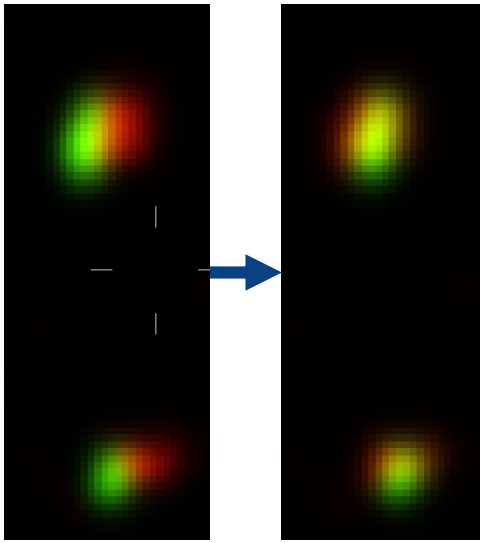
Alignment

Drift correction in x, y, z, t



'Correct 3D Drift' (Parslow 2014)

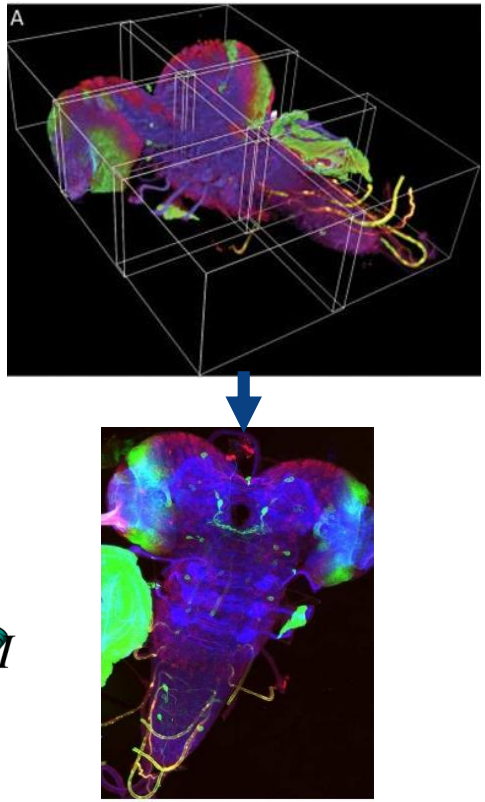
Chromatic shift correction



'TransformJ' (Meijering 2001)

'Chromatic Aberration corrector'

Stitching

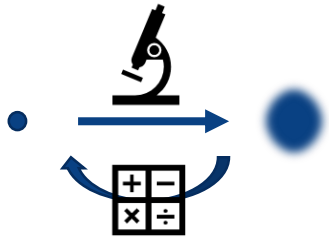


'BigStitcher' (Preibisch 2009)

More details: https://imagej.net/Category:Registration

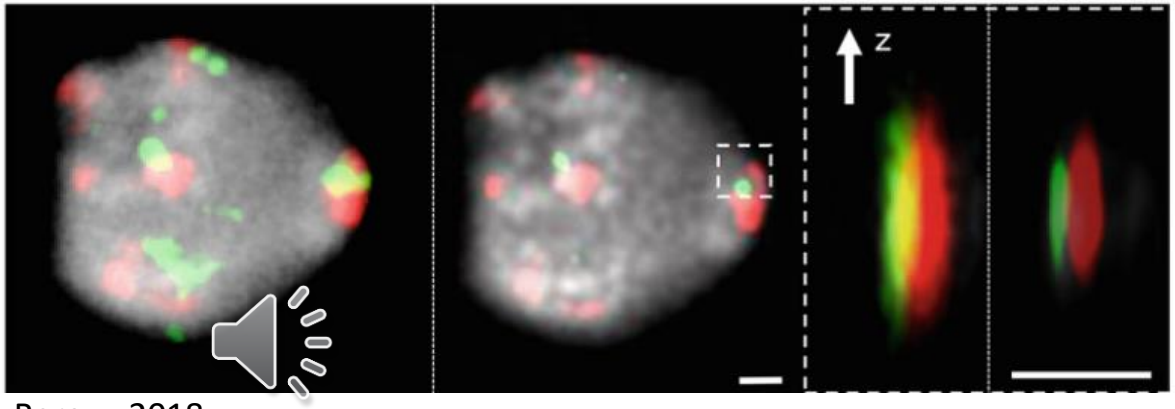
Deconvolution

Mathematical image restoration method

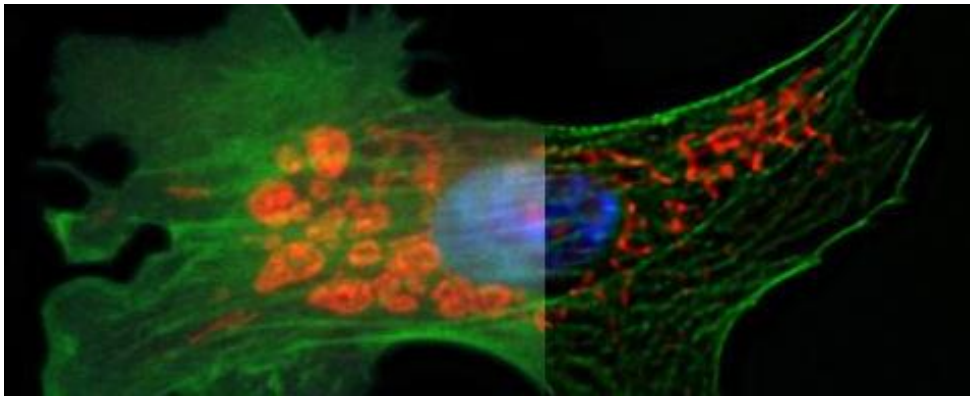


- Increase resolution in x,y,z
- Increase contrast
- Remove noise

Thereby improves the quality of data visualisation and analysis



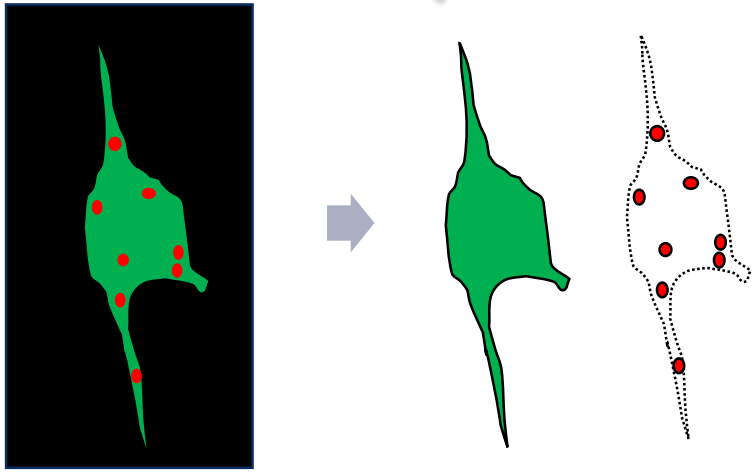
Baroux 2018



www.svi.nl

Image created by Dr. Jeff Tucker and Dr. Holly Rutledge from NIEHS, NIH, USA

Object detection



Raw microscopy image

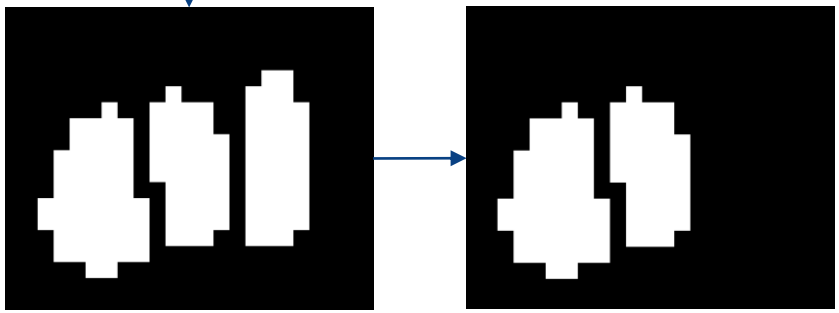
Measurement / Data analysis

Object Detection

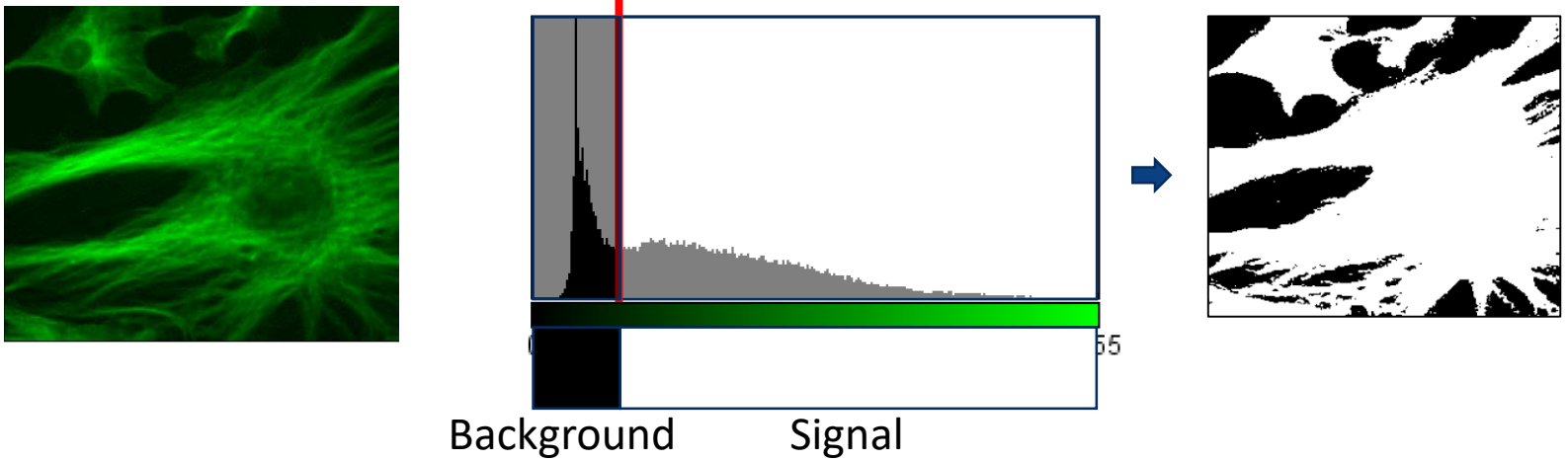
- Pixel segmentation

  - Conventional
  - Machine learning
  - Deep learning
  - Template matching

- Binary processing



Conventional segmentation



- Find threshold using ‘negative control’ image or from the ‘background’ area
- To be ‘objective’, apply same threshold to all images

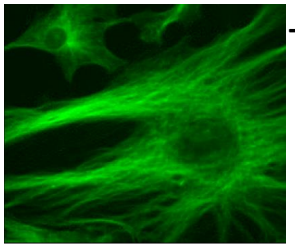
Conventional segmentation

But

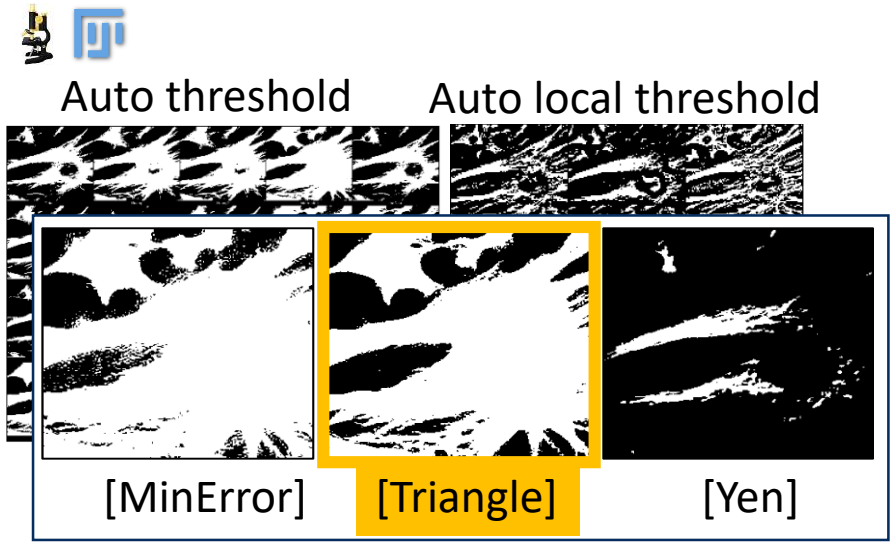
- Many ‘real’ images have different background levels
- Background level may change over time in the same sample in live cell imaging

Auto-threshold algorithms

Huang (1995)	Doyle(1962)
Prewitt (1966)	Shanbhag(1994)
Ridler(1978)	Zack (1977)
Li (1993)	Yen (1995)
Kapur(1985)	Bernsen(1986)
Glasbey(1993)	Soille(2004)
Kittler(1986)	Niblack(1986)
Tsai(1985)	Phansalskar(2011)
Otsu(1979)	Sauvola(2000)
	…any many more



Test

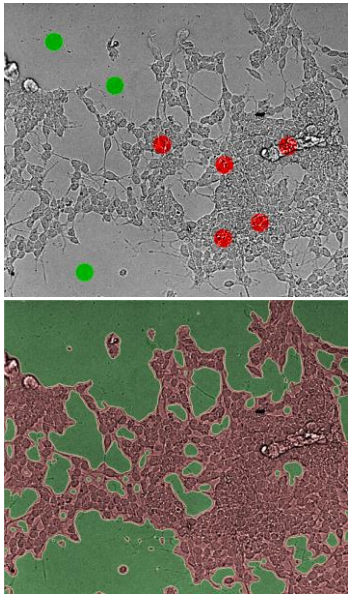


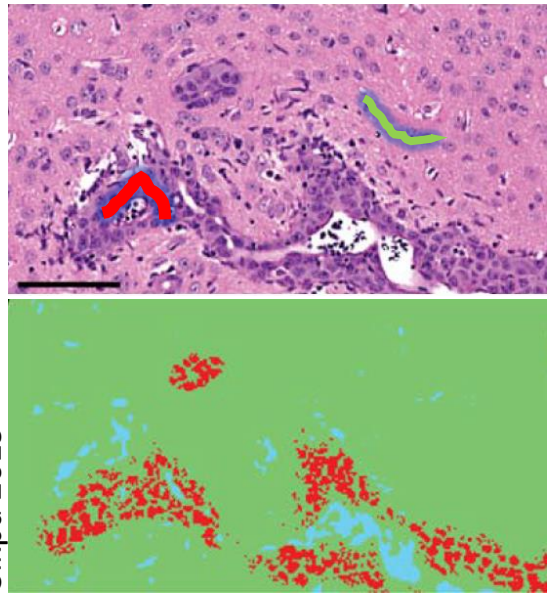
To compensate different background issue,

- Test different Auto-threshold methods
- Apply same auto-threshold algorithm for each image

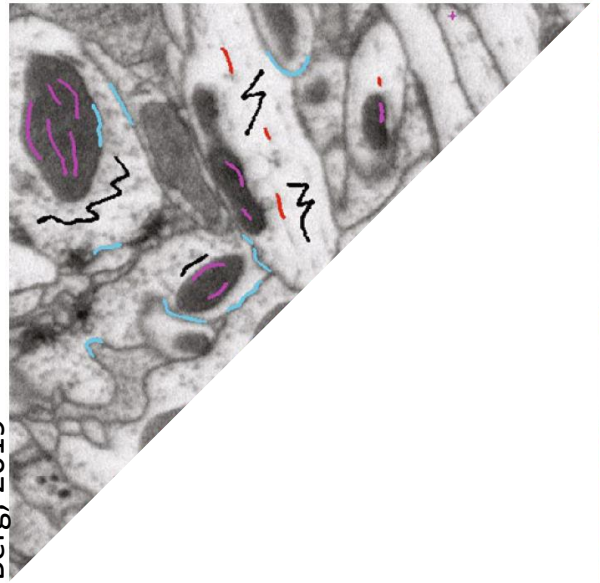
Machine learning

- Thresholding doesn't work well with non-fluorescence images
  e.g. BF, phase contrast, Colour, EM images
- Segmentation using few manual annotations
- Interactive, user-friendly
- No machine learning expertise required





Sikpa 2019



Berg, 2019

Harmony

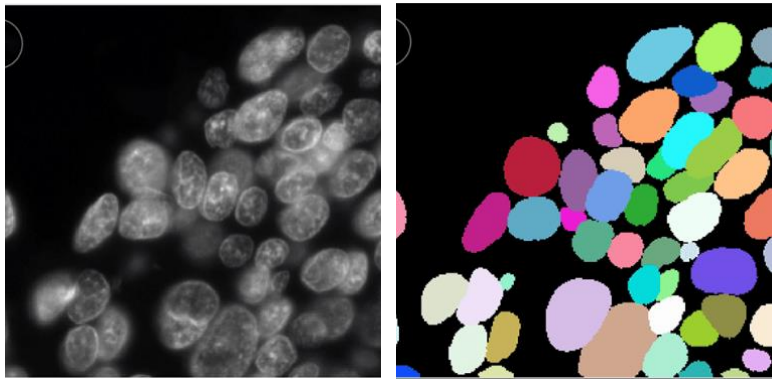
trainable WEKA

Ilastik

Deep learning

- Automatically extracts optimal image features rather than hand-tailored way
- Unsupervised detection but requires manually annotation data and computation
- Some ‘pre-trained’ models for bioimage are accessible via user friendly software

U-net(Falk 2019)(via DeepImageJ)



StarDist (Schmidt 2018)

CellProfiler (U-net, McQuin 2018)

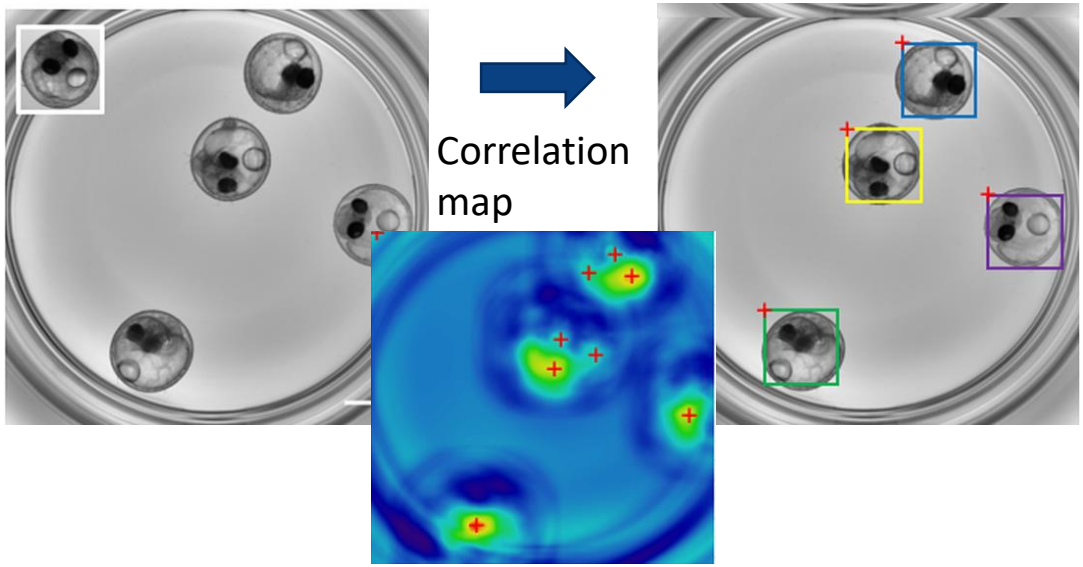
Cellpose (Stringer 2020)

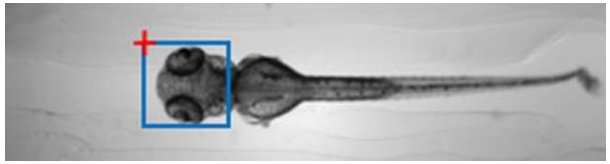
Template matching

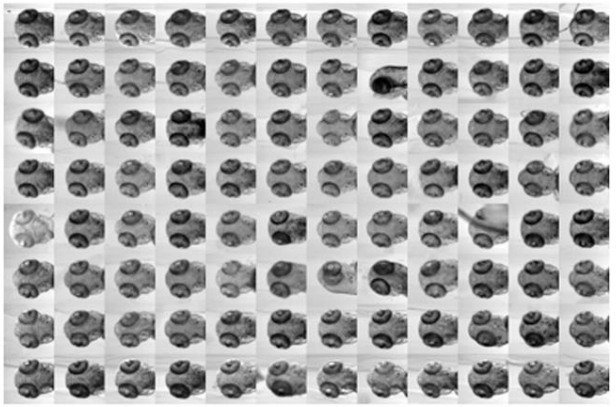
- No manual annotation
- Minimal computation – computes the probability to find one (or several) template images provided by user into a large image
- Good for finding similar structure in time-lapse or TMA
- No programming skill required

Template & Target

Matched image



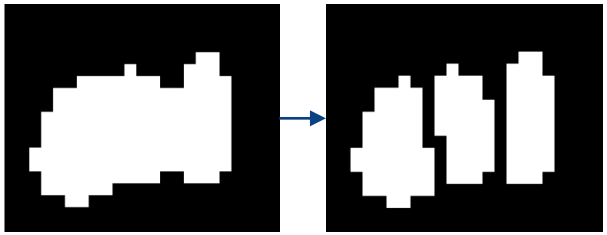




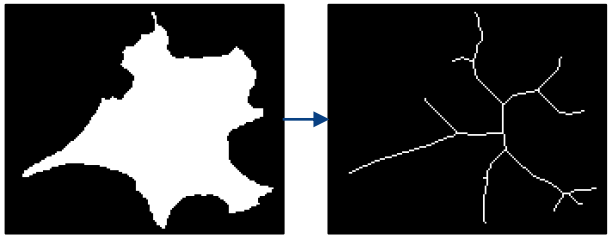
‘MultiTemplateMatching’ (Thomas & Gehrig 2019)

Binary image processing

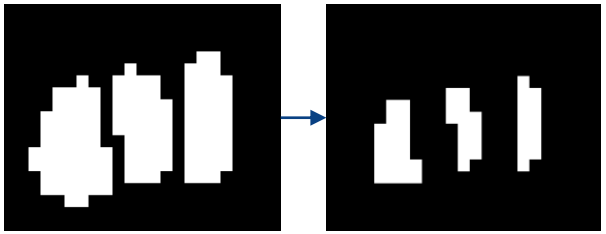
Watershed



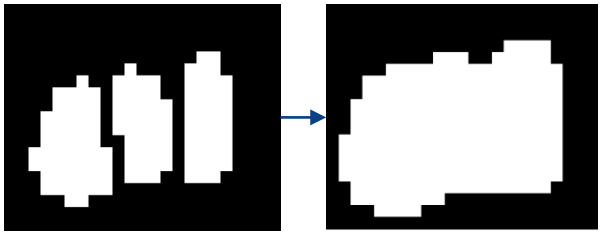
Skeletons



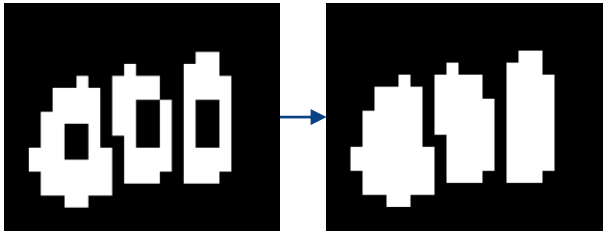
Erode



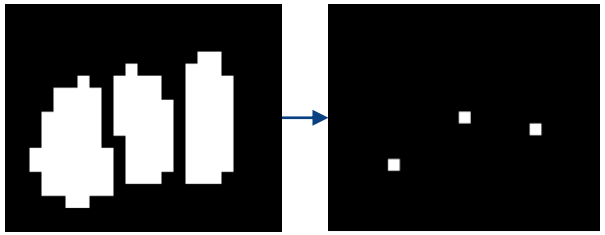
Dilate



Fill Holes



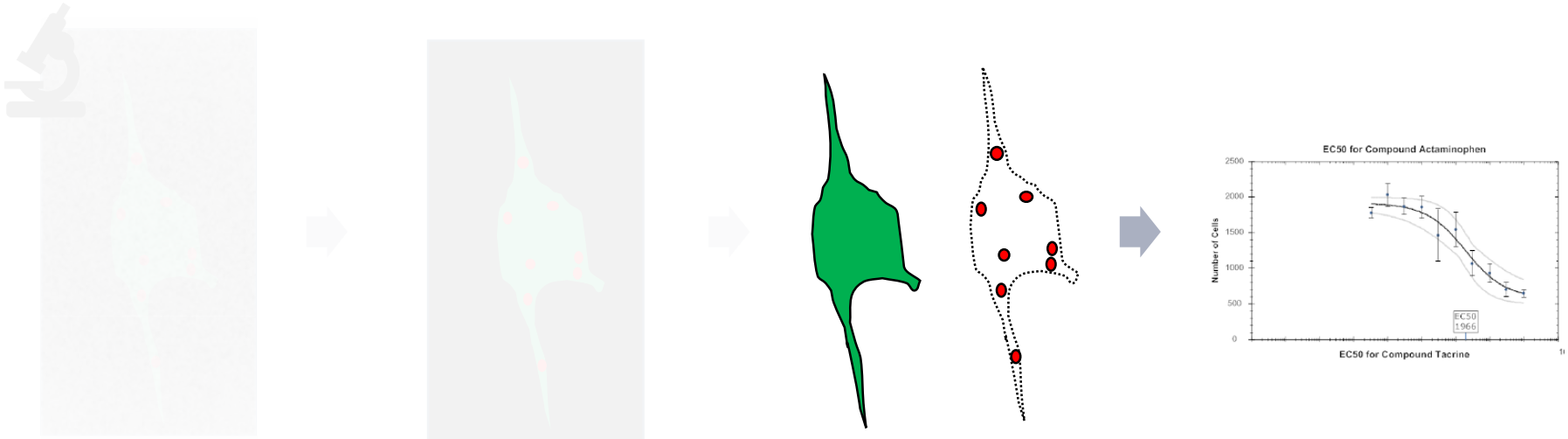
Ultimate eroded points



… many more

Euclidean distance map
Boolean logic

Measurement / Data analysis

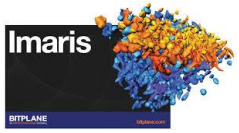


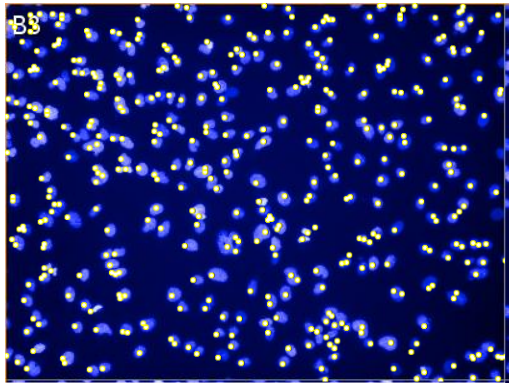
Raw microscopy image

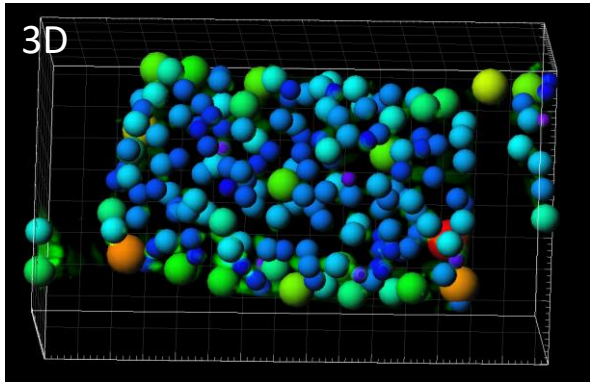
Measurement

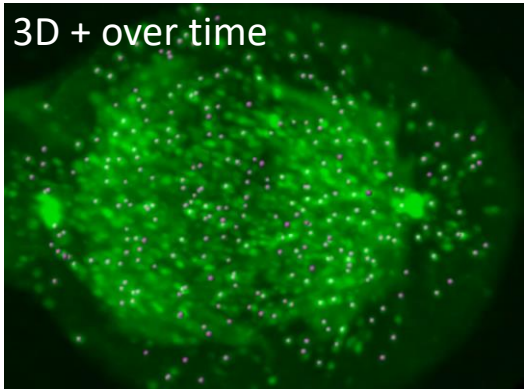
- Number
- Intensity
- Shape
- Distance (spatial analysis)
- Colocalisation / Co-occurrence
- Tracking

Number



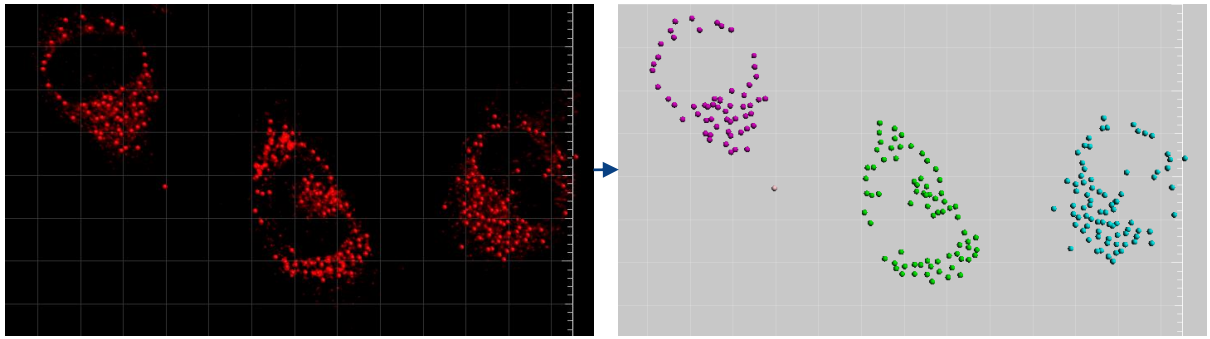






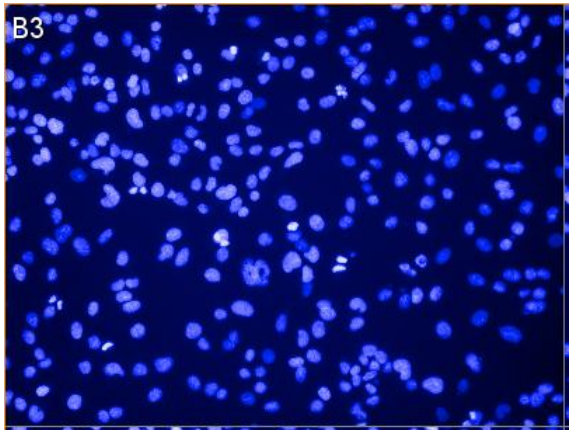
(Yamashita 2015)

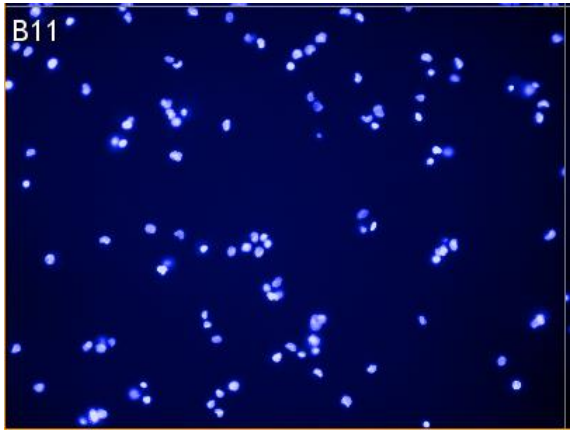
Count per region of interest (ROI)

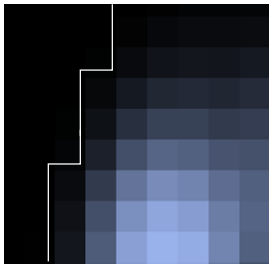


Number of Cell / Measured Area (volume)
Number of Cell / container (ROI)

Intensity

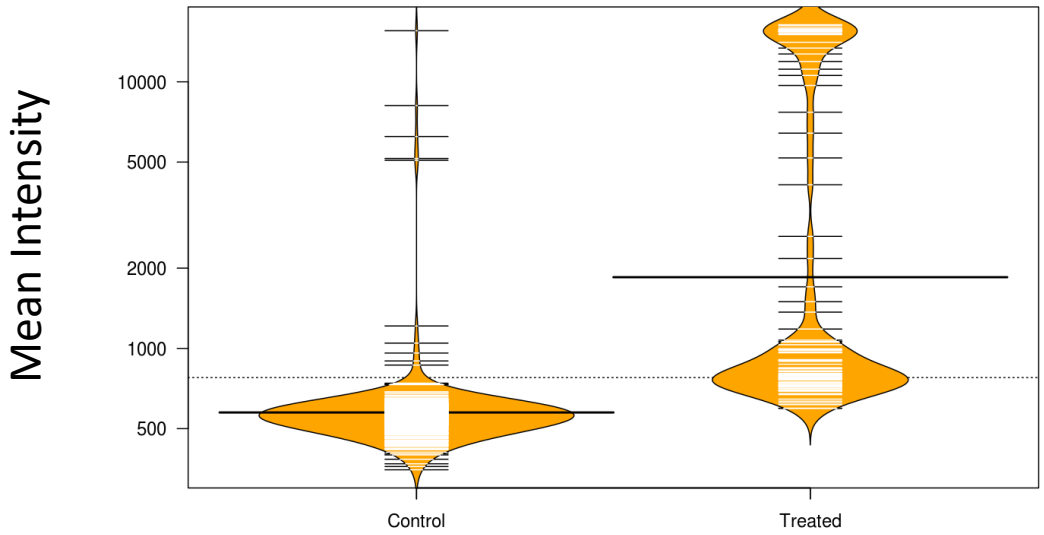






0	0	0	1	4	8	11	11	12
0	0	0	2	10	19	21	21	25
0	0	1	7	24	39	40	38	42
0	0	2	16	45	64	64	57	59
0	0	5	32	76	97	94	81	79
0	1	11	57	112	135	128	105	93
0	1	17	76	139	159	151	121	96
0	1	21	88	154	170	165	128	93

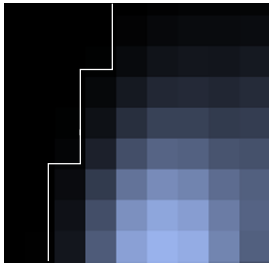
Mean Intensity
= sum intensity / area



Treated cells are brighter

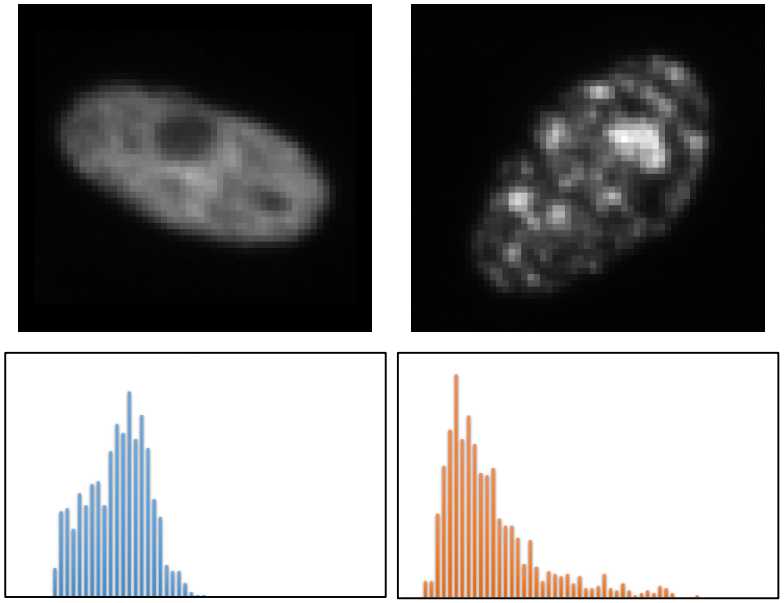
Treated cells shows 2 distinctive populations

Intensity



0	0	0	1	4	8	11	11	12
0	0	0	2	10	19	21	21	25
0	0	1	7	24	39	40	38	42
0	0	2	16	45	64	64	57	59
0	0	5	32	76	97	94	81	79
0	1	11	57	112	135	128	105	93
0	1	17	76	139	159	151	121	96
0	1	21	88	154	170	165	128	93

- Mean
- Minimum
- Maximum
- Median
- Sum
- StdDev

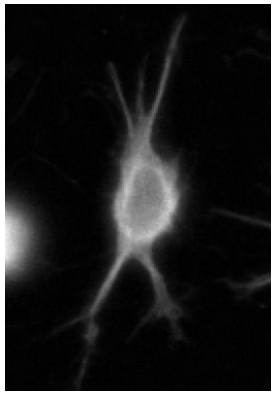


Coefficient of Variation (StdDev/Mean)

0.31

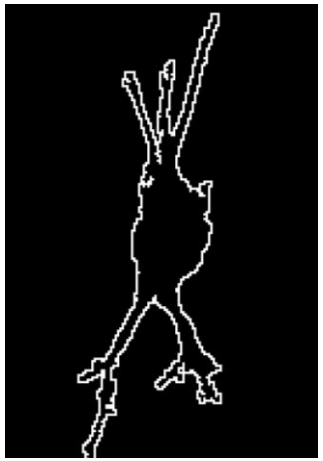
0.55

Shape

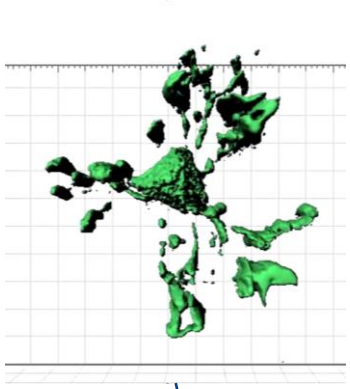


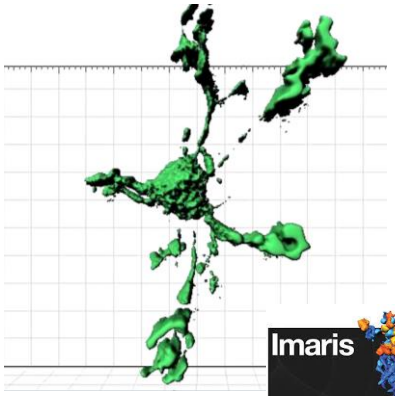
2D: Area
3D: Volume

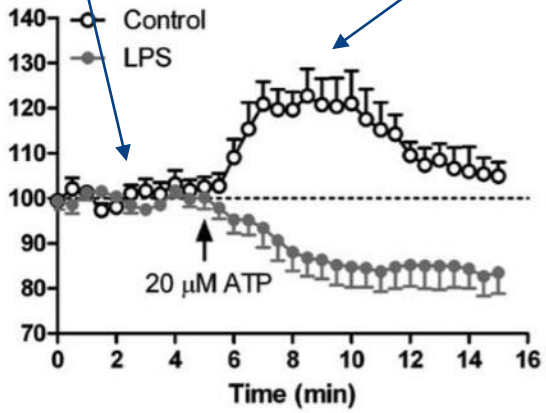
2D: Perimeter
3D: SurfaceArea



Ramification
(% Surface Area:Volume Ratio)



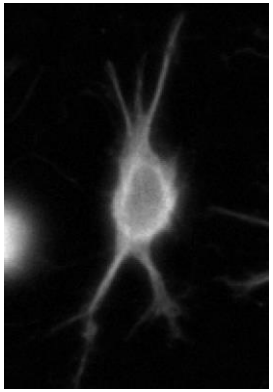


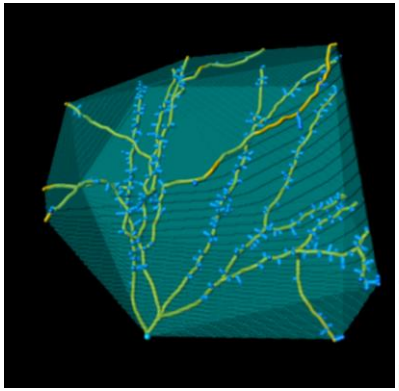
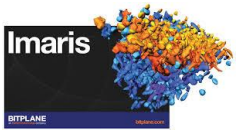


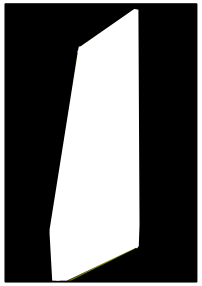
(Gyoneva 2014)

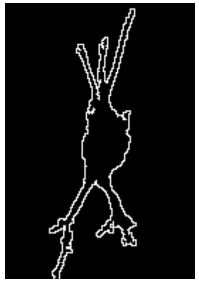
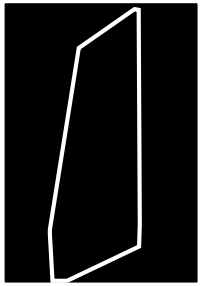
Shape

Convex Hull



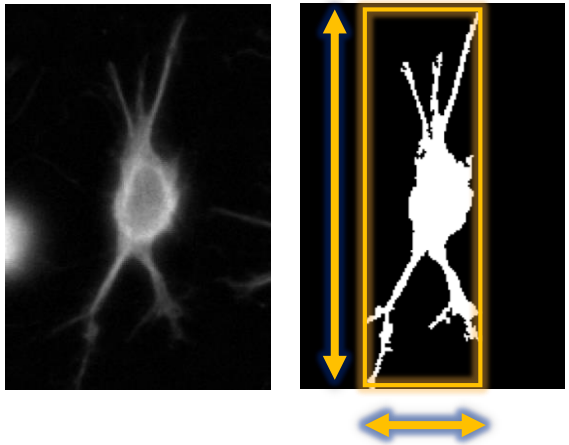



Solidity = : 

Convexity =  : 

Shape

Min bound rectangle

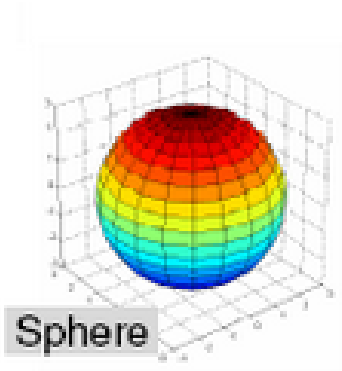


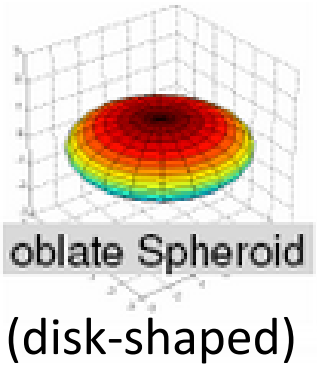
Max Feret Diameter

Aspect Ratio = :

Orientation (Angle) =

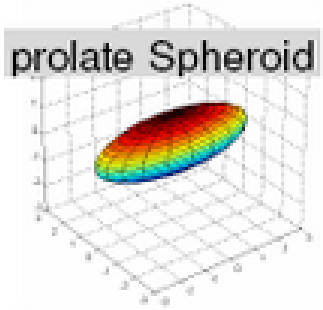
Roundess (sphericity)





(disk-shaped)

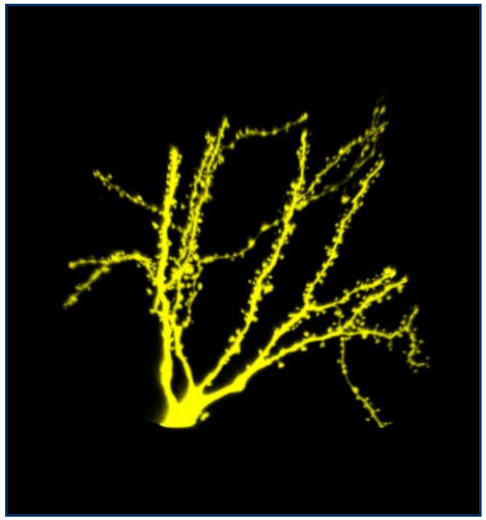
(cigar-shaped)

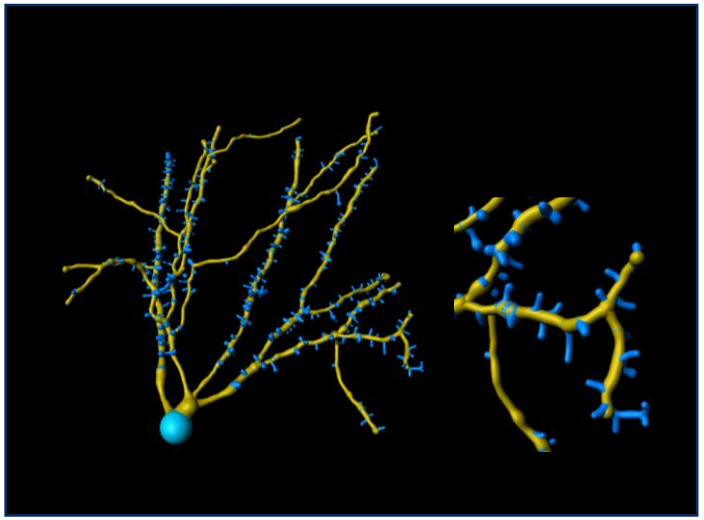


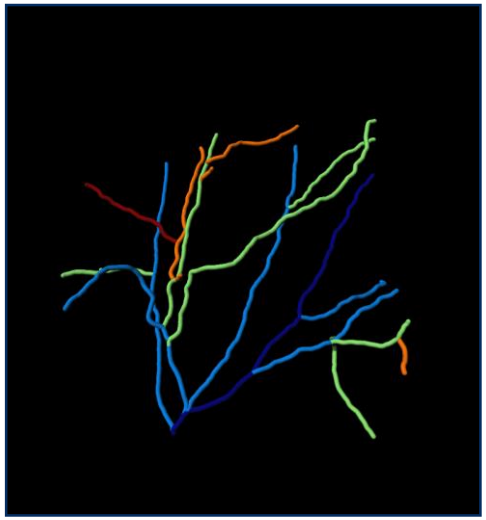
Shape

branch & spine.

Branch level, length, angle..



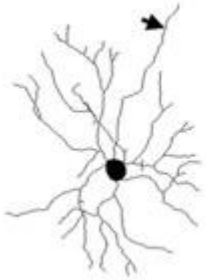


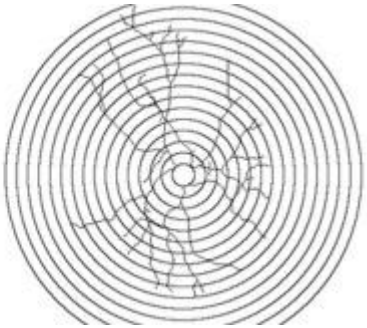


Imaris

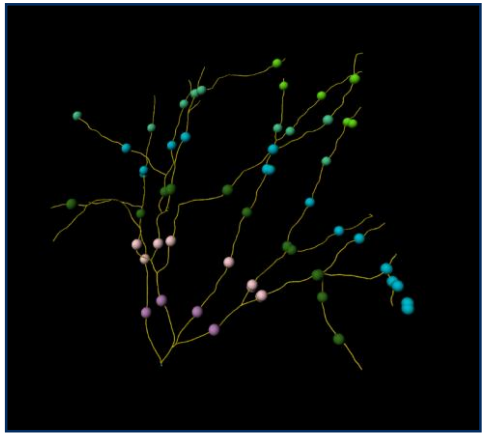
Sholl analysis

Branch number per concentric shell



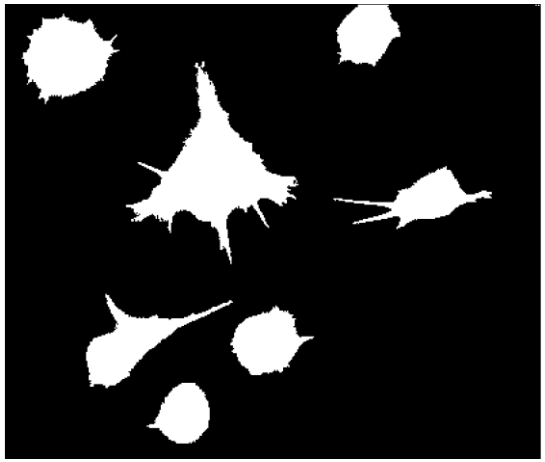


(Binley2014)

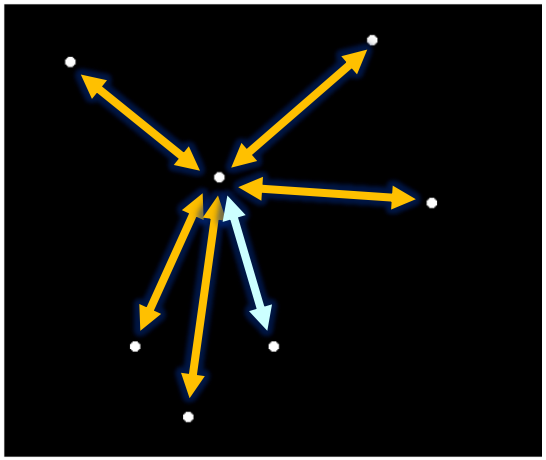


Distance

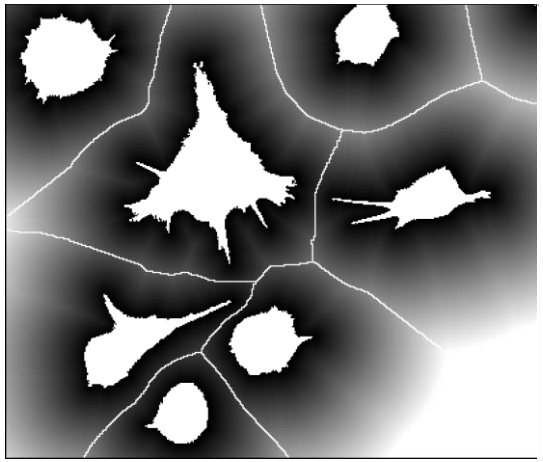
Neighbour relationships ?



Nearest Neighbour Distance (centroid-centroid)

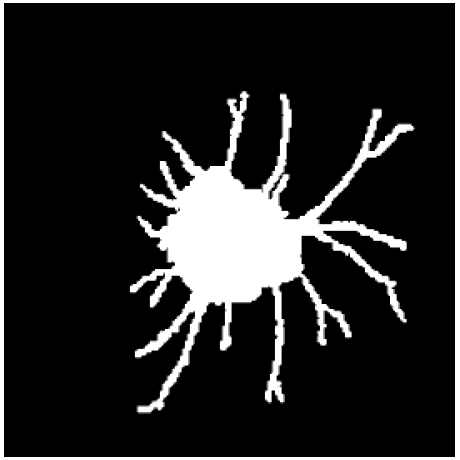


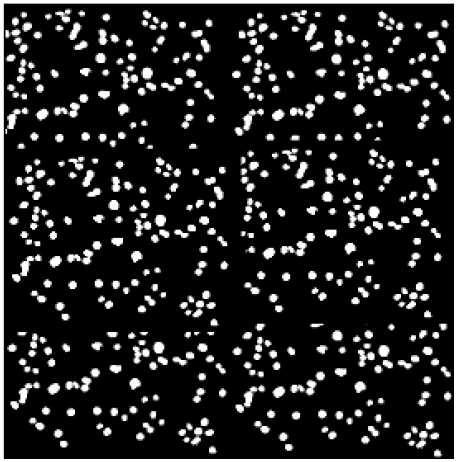
Minimum Separation Distance (edge to edge)

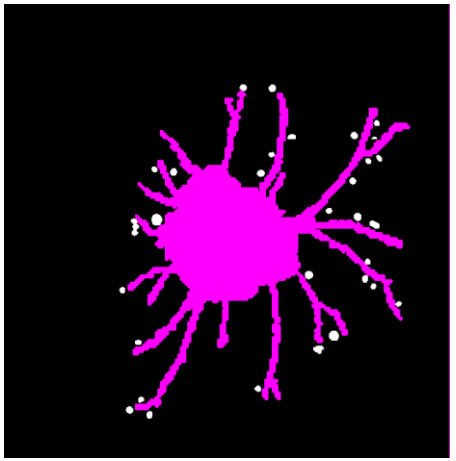


Distance

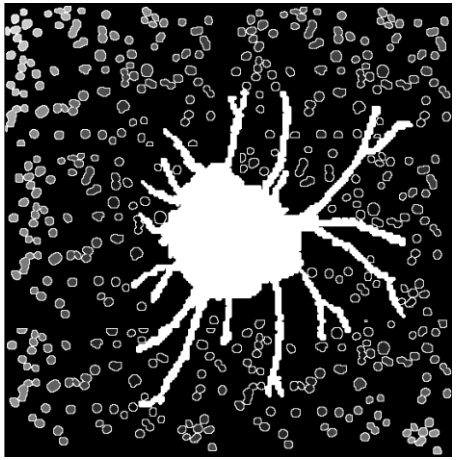
Finding touching object



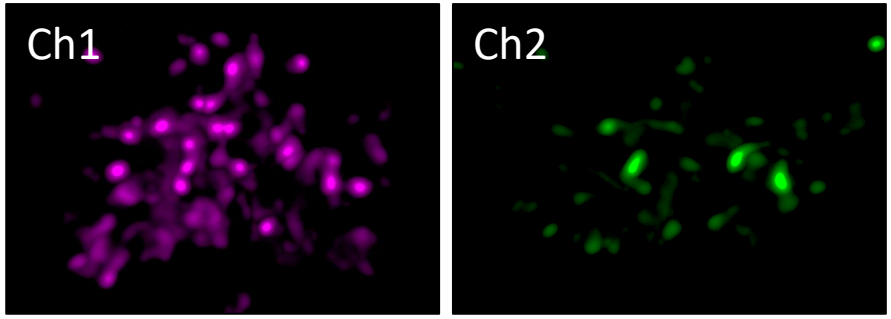




Euclidean distance map

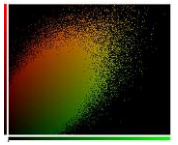


Colocalisation



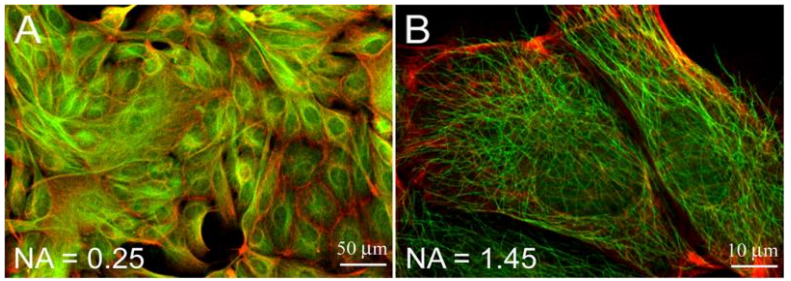
Indication of that two fluorophores are **generally near** each other

molecules are on the *same location*
molecules are *interacting*



- Correlation - Is there a relationship between intensities? (Pearson's)
- Co-occurrence - Are the fluorophores are generally near each other ?
  - Intensity based : Mander's
  - Object based : overlapping area or volume

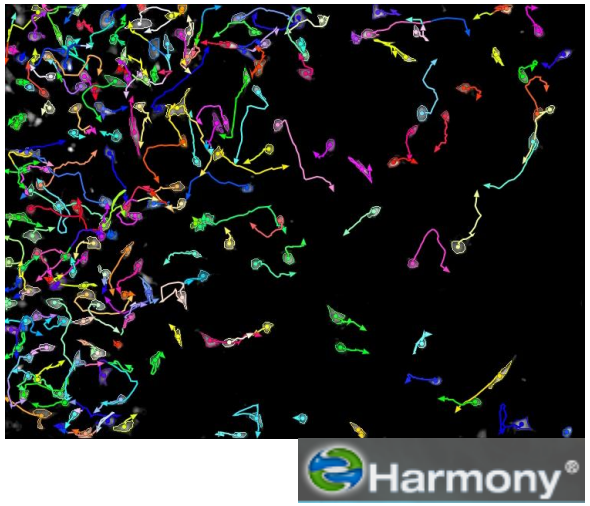
Quantification is heavily dependent on the resolution of acquisition system so careful planning is required

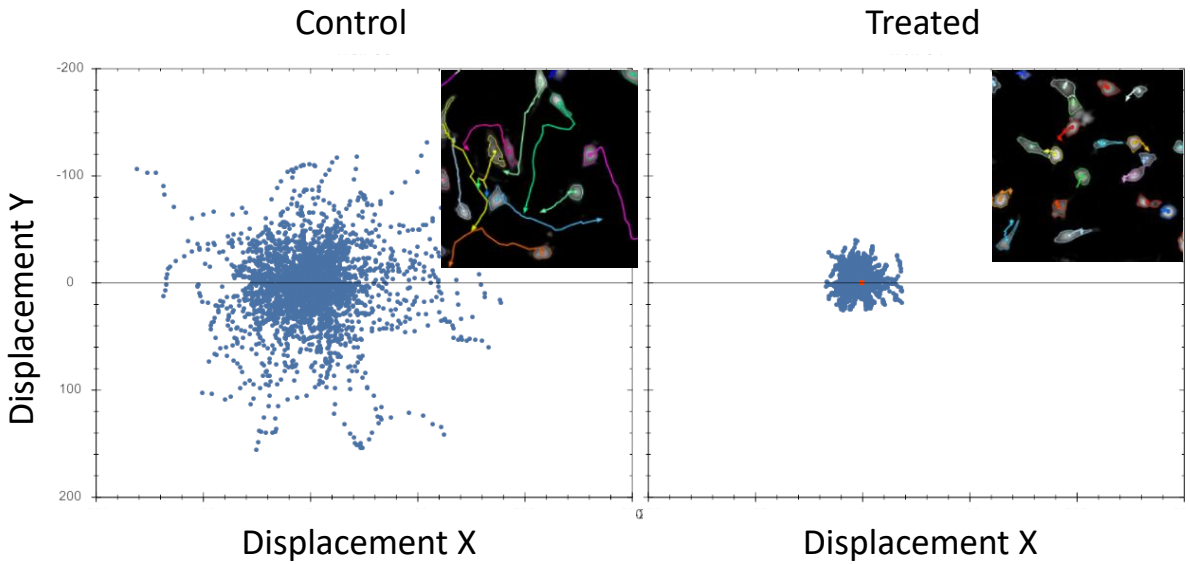


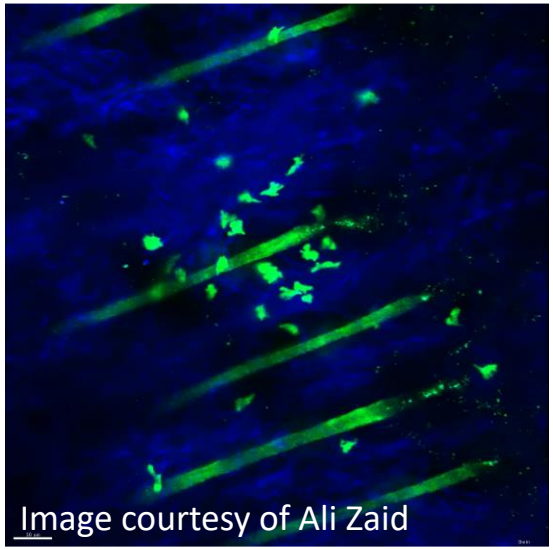
(North 2006)

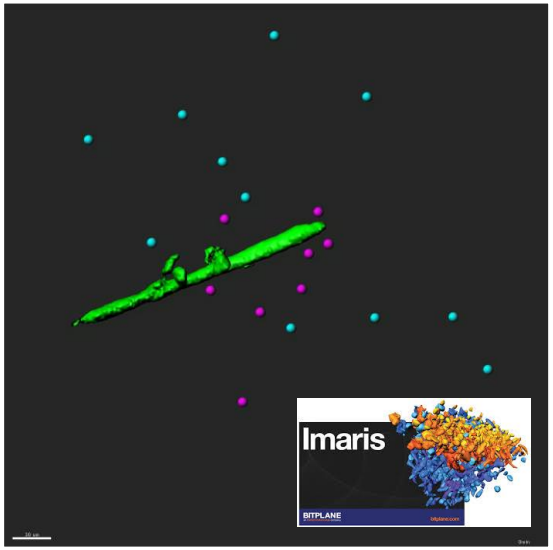
Colocalisation Workshop (https://microscopy.unimelb.edu.au/)

Tracking



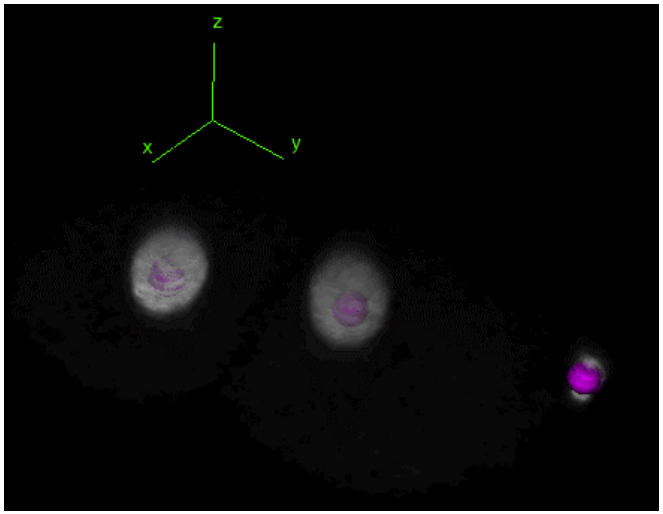






- Speed (µm/s)
- Displacement (µm)
- Direction
- Accumulated distance (µm)
- Straightness
- Duration (s)

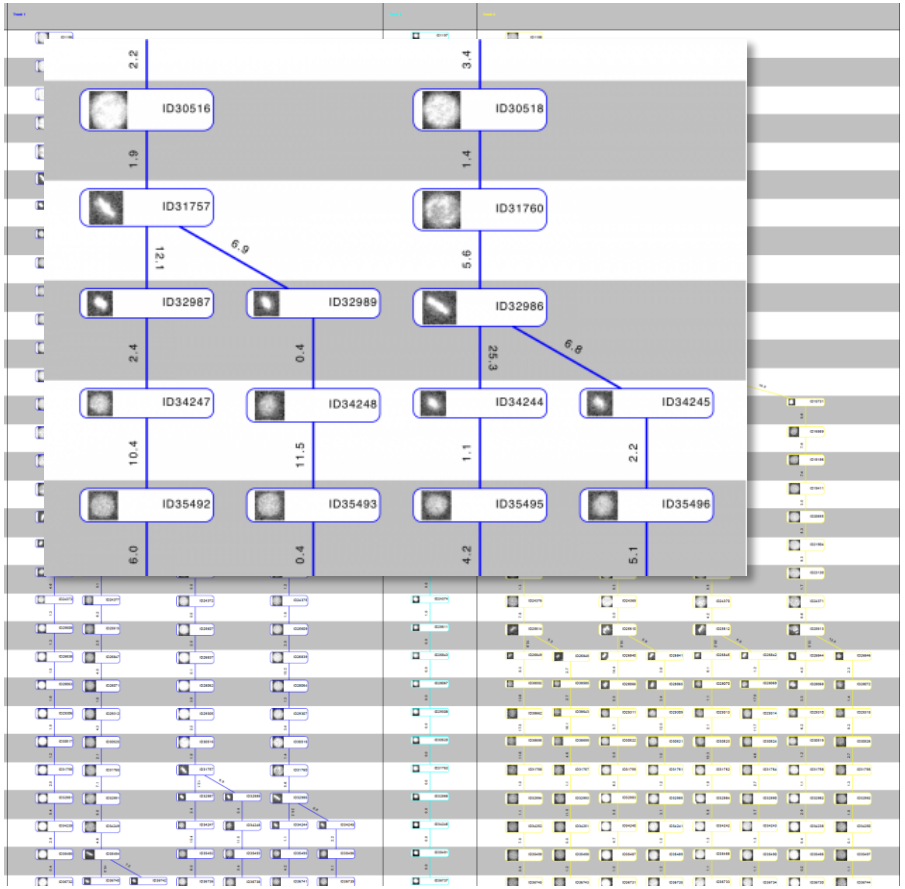
Tracking



TrackMate (Tinevez 2017)

Imaris Lineage tracing

- Lineage tracing



Imaris

Tracking Workshop (https://microscopy.unimelb.edu.au/)

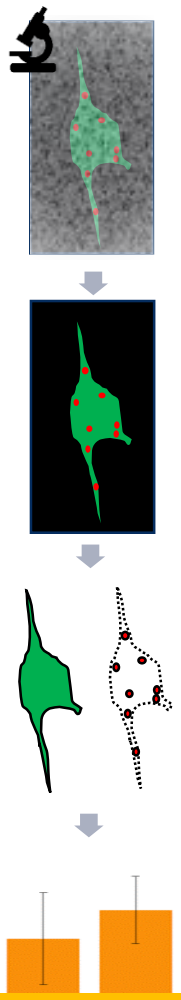
Bioimage analysis workflow

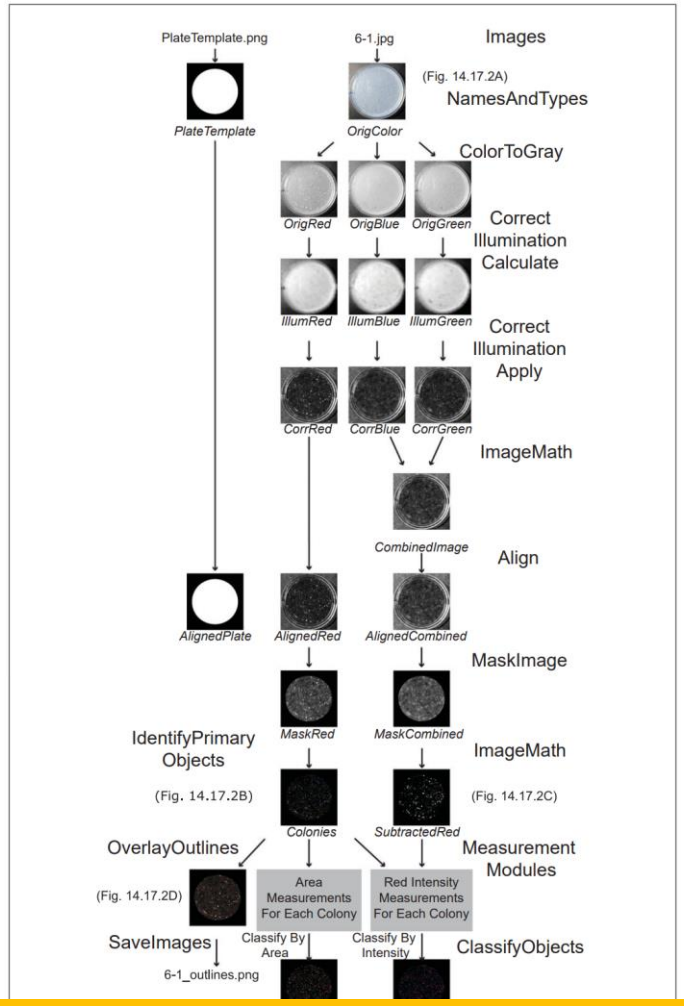
Acquisition

Pre-processing

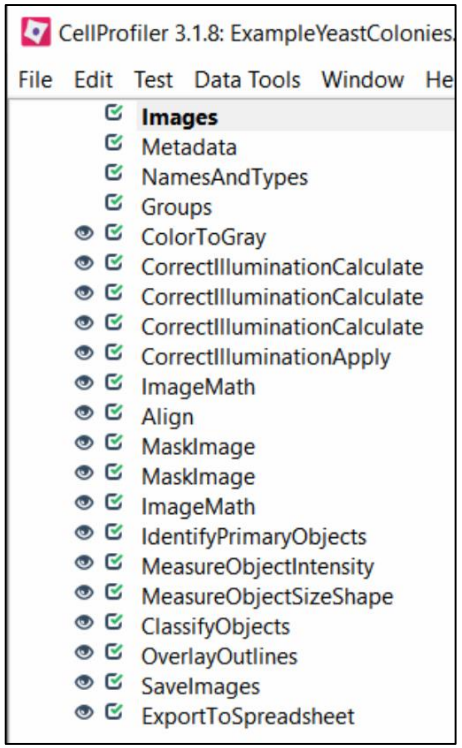
Object Segmentation

Measurement / Data analysis





Cell Profiler



CellProfiler Workshop (https://microscopy.unimelb.edu.au/)

(Bray 2015)

Automate analysis

ImageJ macro

*Process_Folder.ijm

```
#@ File (label = "Input directory", style = "directory") input
#@ File (label = "Output directory", style = "directory") output
#@ String (label = "File suffix", value = ".tif") suffix

// See also Process_Folder.py for a version of this code
// in the Python scripting language.

processFolder(input);

// function to scan folders/subfolders/files to find files with correct suffix
function processFolder(input) {
    list = getFileList(input);
    list = Array.sort(list);
    for (i = 0; i < list.length; i++) {
        if(File.isDirectory(input + File.separator + list[i]))
            processFolder(input + File.separator + list[i]);
        if(endsWith(list[i], suffix))
            processFile(input, output, list[i]);
    }
```

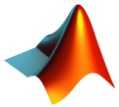
ImageJ Macro writing workshop (https://microscopy.unimelb.edu.au/)

Icy

Python w Scikit-Image

apeer

QuPath



Matlab

Knime

...more

Collaborate with us (bomp-enquiries@unimelb.edu.au)

Must visit places

BOMP website https://microscopy.unimelb.edu.au/

Forum on Bioimage analysis https://forum.image.sc/

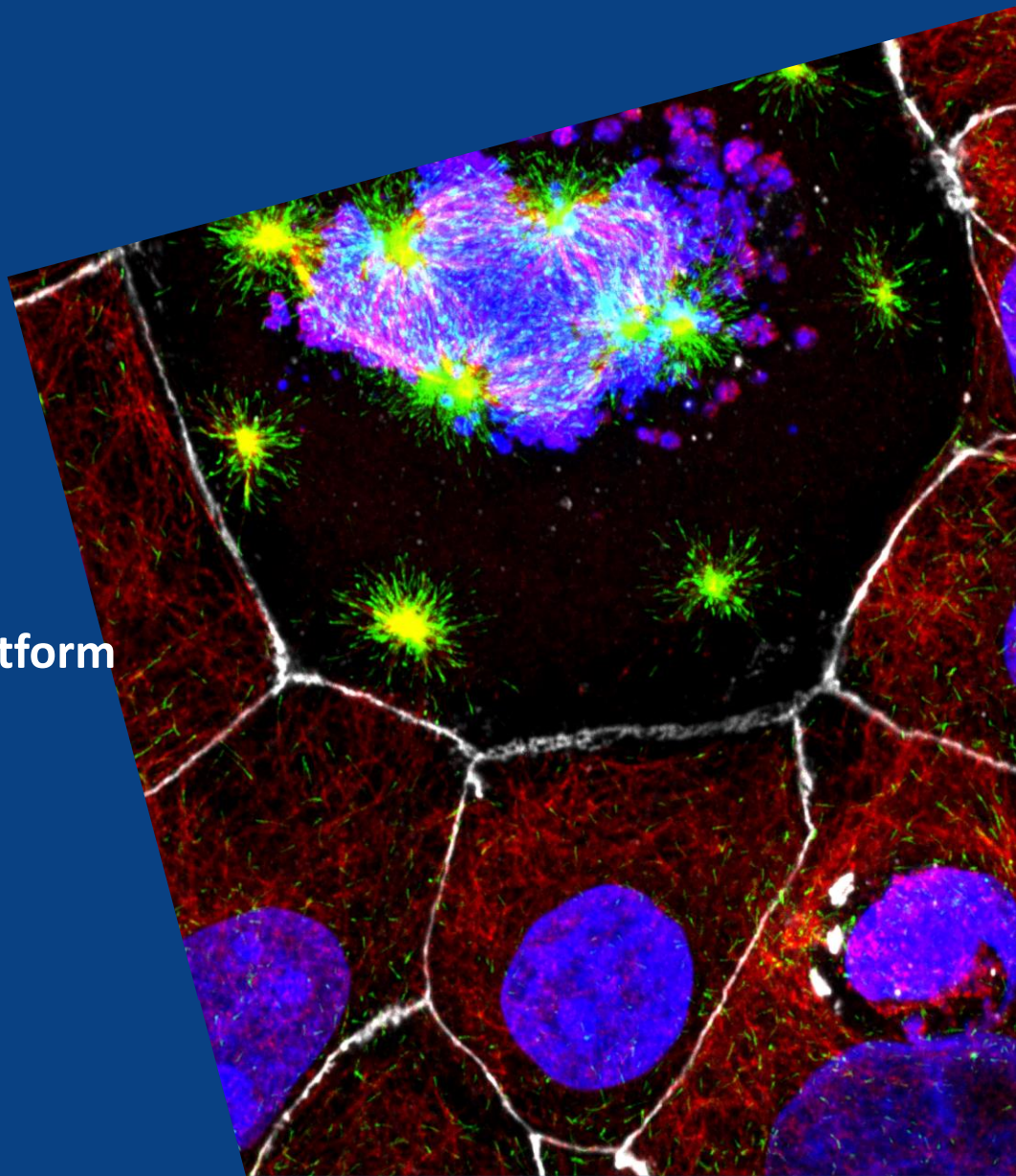
ImageJ learn https://imagej.net/Introduction

Cell Profiler https://cellprofiler.org/

NEUBIAS academy https://neubiasacademy.org/

THE UNIVERSITY OF MELBOURNE

Thank you



Biological Optical Microscopy Platform

bomp-enquiries@unimelb.edu.au
www.microscopy.unimelb.edu.au