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:

- Binary + Digit = Bit
- 1 bit = 0 or 1
Bioimage analysis

Goal:

Obtaining quantifiable information from microscopy image of biological sample

In treated group, cell looks ‘rounder’…

But how much?
Bioimage analysis

Goal:
Obtaining quantifiable information from microscopy image of biological sample
Planning for bioimage analysis
Planning for bioimage analysis

Planning

- Quantification
  - Determine what needs to be measured

- Image acquisition
  - Determine suitable microscope technique

- Sample preparation
  - Prepare sample based on analysis and acquisition plan

Experiment

- Image acquisition
  - Acquire as planned

- Quantification
  - Measure
Planning for bioimage analysis

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

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

Manders M1

Graph showing Manders M1 values for negative, test, and positive samples.
Planning for bioimage analysis

Planning

- Quantification: Determine what needs to be measured
- Image acquisition: Determine suitable microscope technique
- Sample preparation: Prepare sample based on analysis and acquisition plan

Experiment

- Image acquisition: Acquire as planned
- Quantification: Measure

Colocalisation of 2 proteins in mitochondria

Super-resolution to resolve inner and outer membrane of mitochondria

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

Low-resolution? Manders M1

Graphs showing negative, test, and positive values.
Planning for bioimage analysis

**Planning**
- **Quantification**
  - Determine what needs to be measured
- **Image acquisition**
  - Determine suitable microscope technique
- **Sample preparation**
  - Prepare sample based on analysis and acquisition plan

**Experiment**
- **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

Pre-processing

Object Segmentation

Measurement / Data analysis

Raw microscopy image
Image processing software

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

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

As of Jan 2021
Image processing software @ BOMP

Freeware
- ImageJ/Fiji
- CellProfiler™
- ilastik

Commercial
- ZEISS
- LEICA
- NIKON
- Olympus
- Perkin Elmer
- Imaris
- Volocity
- Amira
- MetaMorph®
- Harmony®
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/
Part 2. The Bioimage Analysis Workflow

Acquisition

Pre-processing

Object Segmentation

Measurement / Data analysis

RAW Image
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

- **object**
- **Image formed on microscope (analogue)**
- **Digital sampling**
- **Image acquired (digital)**

Acquire with sufficient **sampling rate**
Raw Microscopy Image

object → Image formed on microscope (analogue) → Image acquired (digital) → Digital sampling

Appropriate resolution for our object
Use higher **Bit depth** for intensity measurement
Raw Microscopy Image

Avoid saturation
Use whole *dynamic range*

Frequency Histogram
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

Raw microscopy image
Pre-processing

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

Fluorescent signal from object

Image formed on microscope
## De-noising

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Image 1</th>
<th>Image 2</th>
<th>Image 3</th>
<th>Image 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Filter</td>
<td>(preserves edge)</td>
<td><img src="bottom-left.png" alt="Image" /></td>
<td><img src="bottom-middle-left.png" alt="Image" /></td>
<td><img src="bottom-middle-right.png" alt="Image" /></td>
<td><img src="bottom-right.png" alt="Image" /></td>
</tr>
<tr>
<td>Gaussian Filter</td>
<td>(smoothing, blurring)</td>
<td><img src="top-left.png" alt="Image" /></td>
<td><img src="top-middle-left.png" alt="Image" /></td>
<td><img src="top-middle-right.png" alt="Image" /></td>
<td><img src="top-right.png" alt="Image" /></td>
</tr>
<tr>
<td>Deep learning</td>
<td></td>
<td><img src="middle-left.png" alt="Image" /></td>
<td><img src="middle-middle-left.png" alt="Image" /></td>
<td><img src="middle-middle-right.png" alt="Image" /></td>
<td><img src="middle-right.png" alt="Image" /></td>
</tr>
</tbody>
</table>

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

Noise2Void (Krull 2019)  
[https://github.com/juglab/n2v](https://github.com/juglab/n2v)
**Background correction**

- **Background subtraction**
  - ‘Rolling Ball’ (Castle and Keller 2007)

- **Shading correction**
  - BaSiC (Peng 2017)

- **Flat Field Correction**
  - Flat field

- **Suppress stripes (Bandpass Filter)**

https://imagej.net/Image_Intensity_Processing#Background_correction
Bleaching correction

Over time or over Z stack

‘Bleach Correction’

‘Bleaching Corrector’
Alignment

Drift correction in x, y, z, t

Chromatic shift correction

Stitching

‘Correct 3D Drift’ (Parslow 2014)
‘Chromatic Aberration corrector’

‘TransformJ’ (Meijering 2001)

‘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

Huygens Image Deconvolution Workshop (https://microscopy.unimelb.edu.au/)
Object detection
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

Tsai (1985)  Phansalskar (2011)
...any many more

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
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

Correlation map

‘MultiTemplateMatching’ (Thomas & Gehrig 2019)
Binary image processing

Watershed

Erode

Fill Holes

Skeletons

Dilate

Ultimate eroded points

Euclidean distance map

Boolean logic

... many more
Measurement / Data analysis
Measurement

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

Count per region of interest (ROI)

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

Mean Intensity
= sum intensity / area

Treated cells are brighter

Treated cells shows 2 distinctive populations
Intensity

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

Coefficient of Variation (StdDev/Mean)

0.31

0.55
Shape

2D: Area
3D: Volume

2D: Perimeter
3D: Surface Area

Ramification
(% Surface Area: Volume Ratio)

(Gyoneva 2014)
Shape

Convex Hull

Solidity = : Convexity = :

Imaris

THE UNIVERSITY OF MELBOURNE
Shape

Min bound rectangle

Max Feret Diameter

Aspect Ratio = : Orientation (Angle) =

Roundess (sphericity)

(cigar-shaped)

(disk-shaped)
Shape

branch & spine.

Branch level, length, angle..

Sholl analysis
Branch number per concentric shell

(Binley2014)
Neighbour relationships? 

Nearest Neighbour Distance (centroid-centroid) 

Minimum Separation Distance (edge to edge)
Distance

Finding touching object

Euclidean distance map
Colocalisation

- Correlation - Is there a relationship between intensities? (Pearson’s)
- Co-occurrence - Are the fluorophores 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.

Indication of that two fluorophores are generally near each other.

Fully interacting molecules are on the same location.

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

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

- Lineage tracing

TrackMate (Tinevez 2017)

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

Acquisition

Pre-processing

Object Segmentation

Measurement / Data analysis

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

(Bray 2015)
Automate analysis

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

ImageJ macro

Icy

Python w Scikit-Image

QuPath

Matlab

apeer

Knime

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/
Thank you

Biological Optical Microscopy Platform

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