FIJI/Image J for Quantification

Hands on session

Dr Paul McMillan
Biological Optical Microscopy Platform

Hands on demonstrations

- FIJI set up
- Line Profile
- Thresholding
- Area of stain
- Cell confluence
- Nuclei counting
- Cell segmentation
- Advanced segmentation
- Intensity over time
- Euclidean Distance Measurements
- Kymogram
- Macro Recording
- Batch Macro Analysis

FIJI set up

- Edit/Options/Colours
  - Foreground: White
  - Background: Black
  - Selection: Yellow
- Process/Binary/Options
  - Iterations: 1, Count: 1
  - Black Background (Tick)
  - Pad edges when eroding (Tick)
  - EDM output: Overwrite
- Edit/Options/Memory and threads
  - Set memory 60-70% of memory
- Plugins/Utilities/Monitor Memory
  - Clear memory as we go
- Plugins/Utilities/Find commands
  - Control L (PC) or Command L (Mac)

Line analysis

- Open Gams.tif, Gams (green) & Gams (red)
- Change line tool to segmented line (right click to change)
- Draw line along filament
- Restore selection (Control + Shift E)
- Plot line profile on gams (green) & gams (red)
  - Analyse/plot profile, Control K (PC) or Command K (Mac)
- Save plot as xls

OPTIONAL ITEMS

- Edit/Selection/Straighten, line width 10 pixels
- Change line colour (Edit/Options/Colours/Selection)
- Change width of line (Edit/Options/Line width)
- Save line as overlay (Image/Overlay/Selection)
  - Control B (PC) or Command B (Mac)

Thresholding

- Open kidney (green).tif
- Image/adjust/auto threshold
  - Select white objects on black background
  - Show threshold values in log window
- Select one that's works best
  - Find threshold values in log window
- Apply selected Threshold
  - Image/Adjust/Threshold
  - Control + Shift T (PC)
  - Command + Shift T (Mac)

Area of stain

- Open kidney (green).tif
- Images/adjust/threshold
- Use method & settings identified in auto threshold
- Analyse/set measurements
  - Area, integrated density, mean gray value, area fraction
  - Limit to threshold
- Measure
  - Analyse/measure
  - Control M (PC)
  - Command M (Mac)
- Right click (or Analyse/Set measurements) to change measurement settings

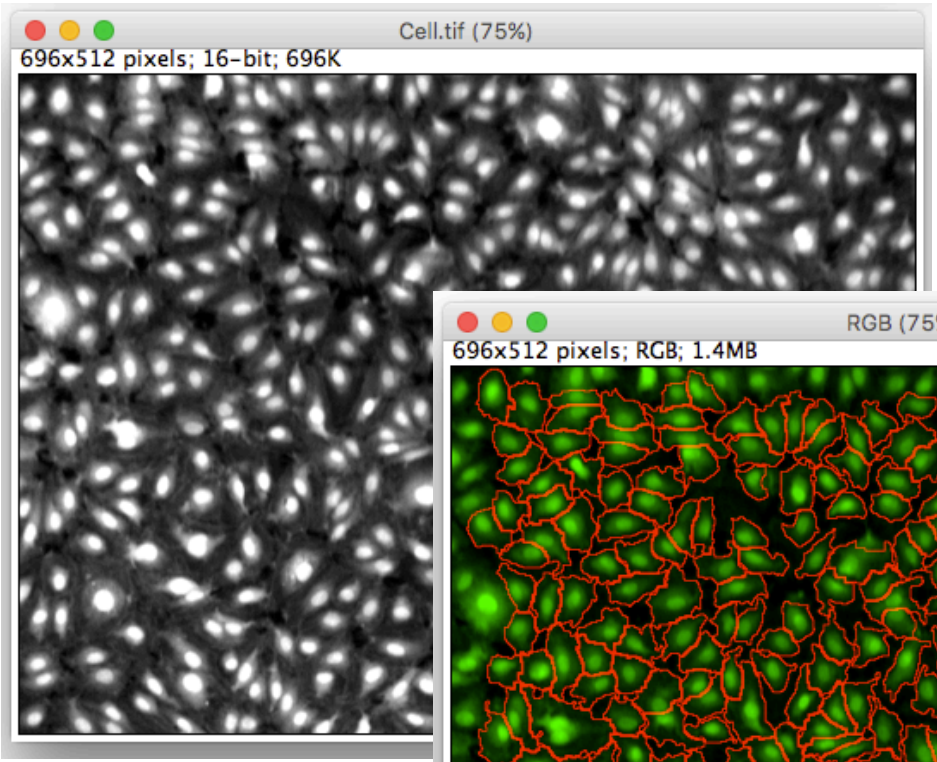
Cell confluency

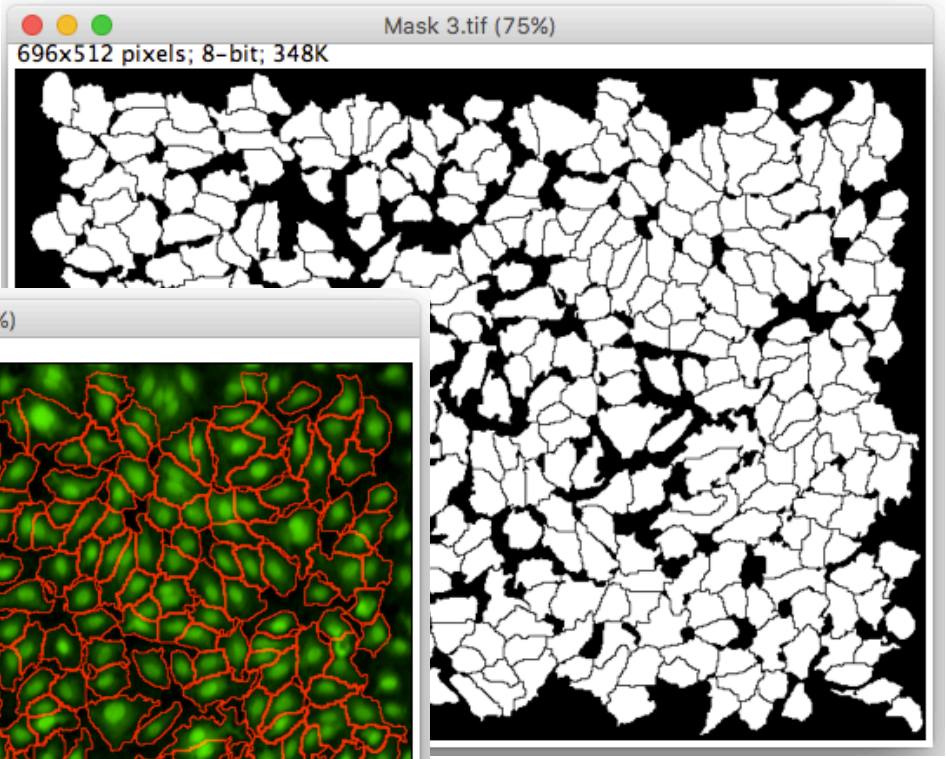
- Open BPAE (green)
- Set threshold
  - Image/Adjust/Threshold
  - Control + Shift T (PC)
  - Command + Shift T (Mac)
- Measure
  - Analyse/Measure
  - Control M (PC)
  - Command M (Mac)
- Right click (or Analyse/Set measurements) to change measurement settings

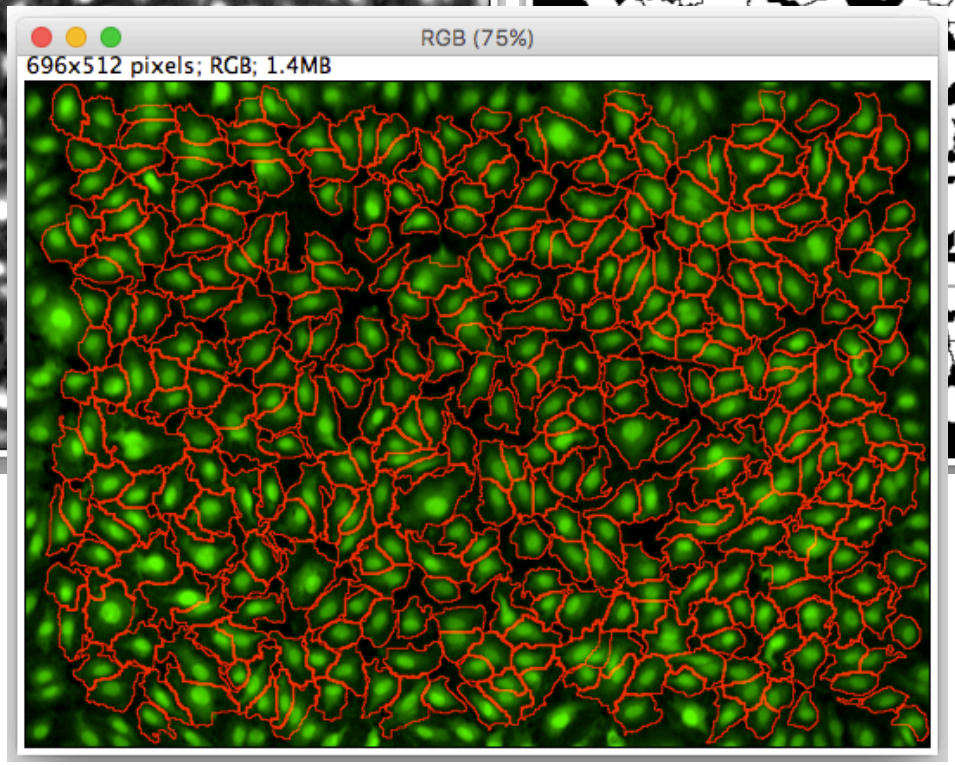
Nuclei Counting

- Open BPAE (blue).tif
- Image/Adjust/Threshold
- Set lower threshold level = 70
- Set higher threshold level = 255
- Apply threshold
- Process Binary/watershed
- Analyze/analyze particles
  - Size = 100-infinity
  - Circularity = 0-1.0
  - Show = outlines
  - Exclude on edges
  - Tick Display results, Summarise, Exclude on edges

Cell segmentation







Cell segmentation

- Open Cell.tif (from Segmentation) & Duplicate
- **Watershed (Save final image as MASK 1.tif)**
  - Process/Find Maxima (Noise = 400, exclude on edges, segmented particles)
- **Whole cell stain (Save final image as MASK 2.tif)**
  - Threshold duplicated image (min = 388, max 4095) but don't apply yet
  - Process/smooth & apply threshold
- **Cell outlines (Save final image as MASK 3.tif)**
  - Process/Image Calculator Mask 1 **AND** Mask 2
  - Analyze/Analyze Particles (Size = 250 – Inf, exclude on edges, show masks)
  - Invert LUT
  - Process/Binary/Fill Holes
- **Analyse the images**
  - Analyze/Set Measurement (Area, Shape, Int Den, Mean, Perimeter, Ferets, Display label. REDIRECT to Cell.tif
  - Analyse Particles (size = 250-infinity, Show = outlines, display, clear, summarize, exclude on edges)
  - Try as above, but also select ROI manager

Advanced segmentation

Cytoplasmic masks (Cells minus nucleus)

- Open Nuclei.tif
- Threshold & create binary (Save as Mask N)
- Process/Binary/Watershed
- Process/Image calculator/ Mask 3 SUBSTRACT MASK N

Perinuclear mask

- Open Mask N & Duplicate
- Process/Binary/Dilate (on Mask N-1.tif) & Process/Binary/Watershed
- Process/Binary/Erode (on Mask N.tif)
- Process/Image calculator/Mask N-1 SUBTRACT Mask N

Intensity over time

- Open Calcium flux.tif
- Draw ROI on bottom right cell
- Analyze/Set Measurement
  - Mean Gray value
  - Display label
- Image/Stacks/Plot Z axis Profile
- Repeat on background

- Analyze/Tools/ROI manager
- Add multiple ROIs
- Show all
- Select More, Multi Measure, Measure all 50 slides, one row per slice

Euclidean distance measurement

- Open Nuclei.tif, apply threshold & create binary image
- Process/Binary/Options
  - Configure EDM to 16 bit
- Edit/Invert
- Process/Binary/Distance Map
- Apply “16 colours” LUT
- Analyze/Set Measurements
  - Mean gray value, limit to threshold, display label
- Threshold to select background (use 1-29 threshold)
- Analyze/Measure
- Average distance = 8.098 Pixels (read out is always in pixels)
- Calculate distance in microns on calibrated images
  - Image/Properties, Control + Shift P (PC), Command + Shift P (Mac)
  - Covert using pixel dimensions

Kymogram

- Open tracking.tif from Kymogram
- Image/Colour/Split channels
- Draw line form ROI tools (Hold shift to keep it straight)
- Select channel two and restore selection (Ctrl + Shift E)
- Select channel 1, Analyze/Multi Kymporaph/Multi Kymograph
- Set line width to 29 (must be an odd number)
- Image/Rename, rename channel 1
- Repeat Kymograph (as above) for Channel 2
- Merge Channels (Channel 1 = green, Channel 2 = Red)

Macro Recording

Macro Recording:

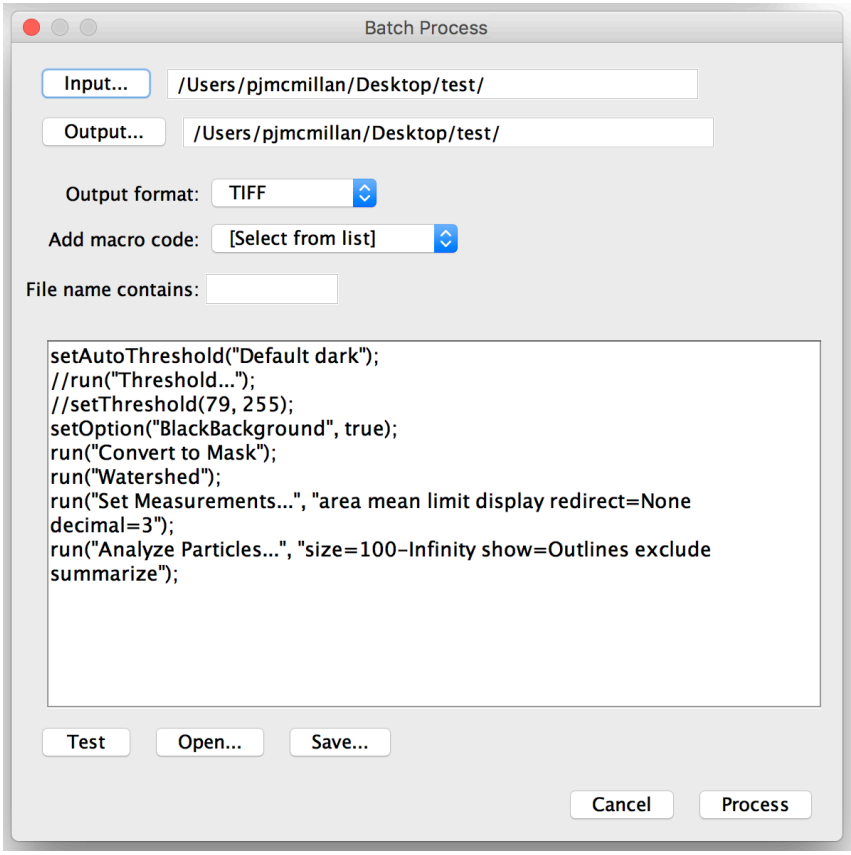
- Plugins/Macros/Record
- Repeat a simple analysis used earlier (e.g. Nuclei Counting)
  - Remove any mistakes or unwanted steps (e.g. “Select Window” for image opening)
- Rename macro & press Create
- Close all open images and open an image to test (e.g. BPAE (blue.tif for the nuclei counting)
- Hit Run and it should perform the steps automatically
- File/Save (or Save as) to define where to save it to
  - Always save with .ijm file extension for FIJI to read the macro

Macro Running:

- Plugins/Macros/Run to use the Macro again

Batch Macro Analysis

- Process/Batch/Macro
- Copy macro details into the dialogue box
- Define input folder (where the images to be analysed are)
- Define output folder (if you are saving output images)
- File name contains
  - Filter images analysed based on file name (e.g. only images with ch01)
- Use test to make sure the code works
- Press process for all images to be analysed



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