Quantitative microscopy

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FIJI - IMAGEJ

Line analysis

Aims: Plot image intensity along a line ROI

1. Open Gams.tif, Gams (green) & Gams (red)
2. Change line tool to segmented line (right click to change)
3. Draw line along filament
4. Restore selection (Control + Shift E)
5. Plot line profile on gams (green) & gams (red)
   - Analyse/plot profile, Control K (PC) or Command K (Mac)
6. 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)

Adapted from "Fundamentals of Image Quantification" Prof McMillan - University of Melbourne

Thresholding

Aim: Identify areas in an image based on intensity

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

Use selected Threshold (Dont hit apply)

- Image/Adjust/Threshold
- Control + Shift T (PC)
- Command + Shift T (Mac)
- Use auto feature

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Area of stain

Aim: Quantify the area in an image above a given intensity value

1. Open kidney (green).tif
2. Images/adjust/threshold
3. Use method & settings identified in auto threshold
4. Analyse/set measurements

- Area, integrated density, mean gray value, area fraction
- Limit to threshold

5. Measure

- Analyse/measure
- Control M (PC)
- Command M (Mac)

6. Right click (or Analyse/Set measurements) to change measurement settings

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Cell confluency

Aim: Calculate cell confluency of an image using thresholding

1. Open BPAE (green)
2. Set threshold (don't hit apply)

- Image/Adjust/Threshold
- Control + Shift T (PC)
- Command + Shift T (Mac)
- Use over/under rather than red threshold to visualize better

3. Measure

- Analyse/Measure
- Control M (PC)
- Command M (Mac)

4. Right click (or Analyse/Set measurements) to change measurement settings

Nuclei Counting

Aim: Automated counting of objects in an image

1. Open BPAE (blue).tif
2. Image/Adjust/Threshold (Set lower threshold level = 70, higher threshold level = 255)
3. Apply threshold
4. Process Binary/watershed
5. Analyze/analyze particles

- Size = 100-infinity
- Circularity = 0-1.0
- Show = outlines
- Exclude on edges
- Tick Display results, Summarise, Exclude on edges

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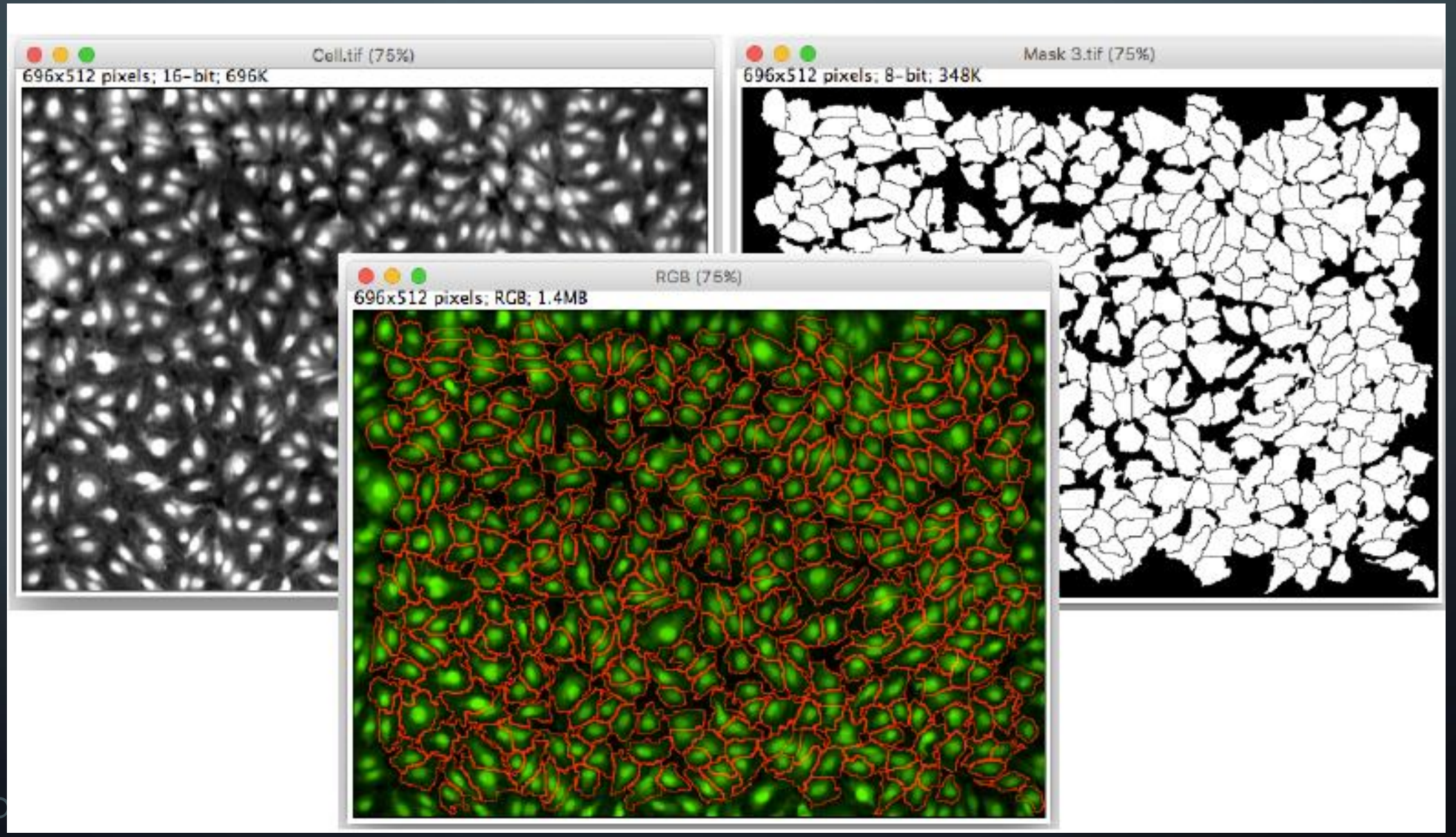
Cell segmentation



Adapted from "Fundamentals of Image Quantification" Prof McMillan - University of Melbourne

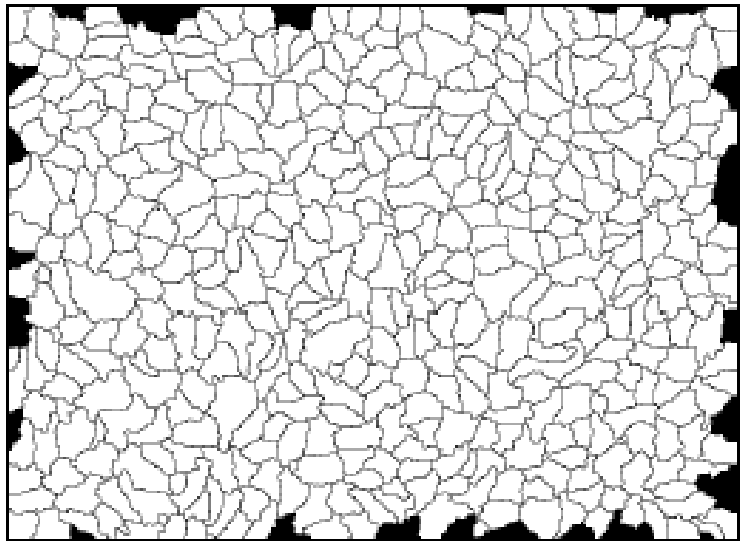
Cell segmentation

Aim: Quantify multiple measurements from individual cells in a crowded image

Open Cell.tif (from Segmentation) & Duplicate

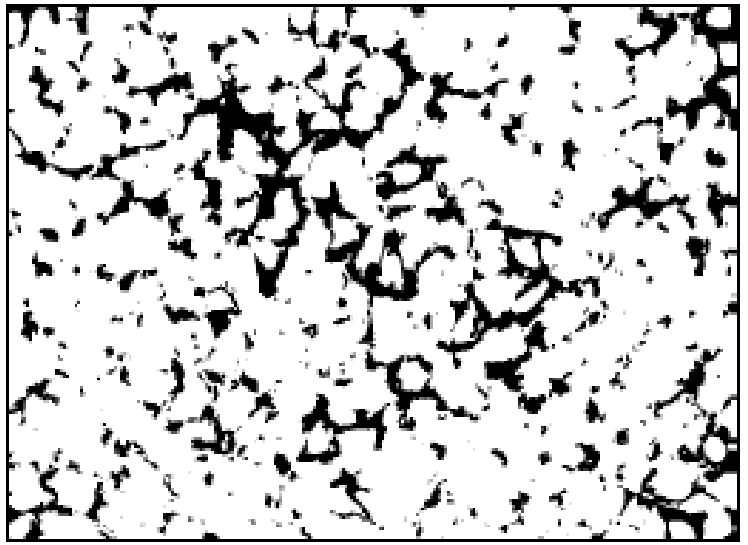
Mask 1: Watershed

- Process/Find Maxima (Noise = 400, exclude on edges, segmented particles)
- Save final image as MASK 1.tif



Mask 2: Whole cell stain

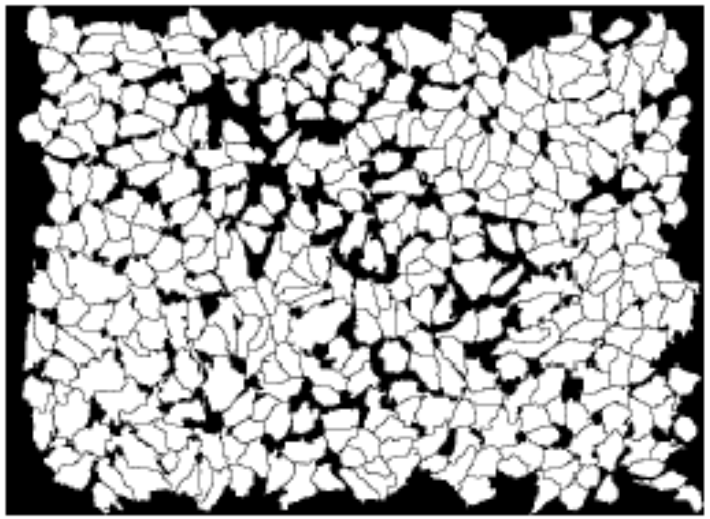
- Threshold duplicated image (min = 388) but don't apply yet
- Process/smooth
- Apply threshold
- Save final image as MASK 2.tif



Cell segmentation

Mask 3: Cell outlines

- Process/Image Calculator Mask 1 **AND** Mask 2
- Analyze/Analyze Particles (Size = 250 – Inf, exclude on edges, show masks)
- Invert LUT (Image/LUT/Invert LUT)
- Process/Binary/Fill Holes
- Save final image as MASK 3.tif



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 "add to manager" (ROI Manager)

Adapted from "Fundamentals of Image Quantification" Prof McMillan - University of Melbourne

Aim: Continue to use image masks to further quantify objects in images

Cytoplasmic masks (Cells minus nucleus)

1. Open Nuclei.tif
2. Threshold & create binary
3. Process/Binary/Watershed
4. Save as Mask N
5. Process/Image calculator/ Mask 3 **SUBTRACT** MASK N

Perinuclear mask

1. Open Mask N & Duplicate, rename one Dilate & other Erode
2. Process/Binary/Dilate (on Dilate) & Process/Binary/Watershed
3. Process/Binary/Erode (on Erode.tif)
4. Process/Image calculator/Dilate **SUBTRACT** Erode

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Intensity over time

Aim: Measure intensities in a timelapse image

Open Calcium flux.tif

Draw ROI on bottom right cell

Analyze/Set Measurement

- Mean Gray value
- Display label, untick "limit to threshold"

Image/Stacks/Plot Z axis Profile

Repeat on background

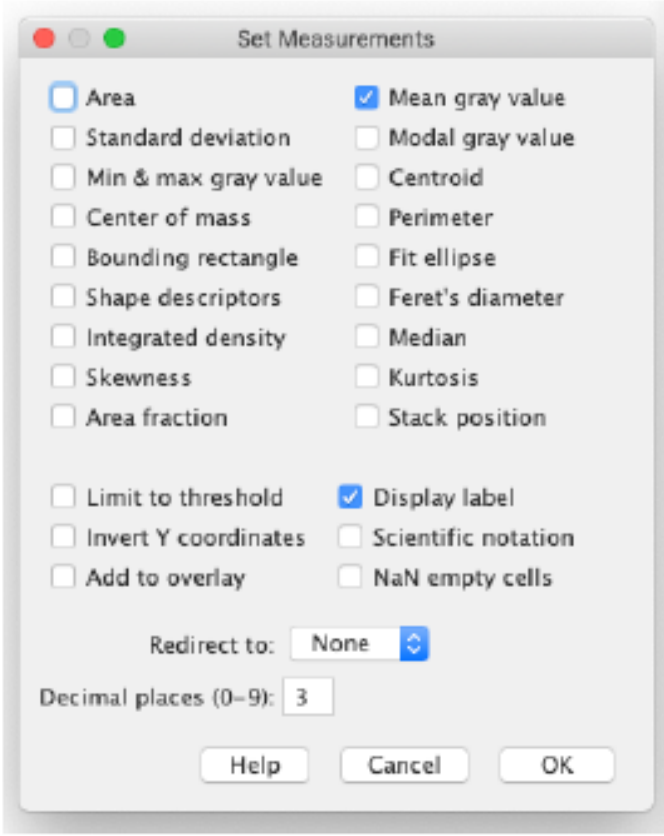
For multiple ROI's per image:

Analyze/Tools/ROI manager

Add multiple ROIs

Show all

Select More, Multi Measure, Measure all 50 slides, one row per slice



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Euclidean distance measurement

Aim: Measure the average distance between objects in an image

1. Open Nuclei.tif, apply threshold & create binary image
2. Process/Binary/Options (Configure EDM to 16 bit)
3. Edit/Invert
4. Process/Binary/Distance Map
5. Apply "16 colours" LUT
6. Analyze/Set Measurements (Mean gray value, limit to threshold, display label)
7. Threshold to select background (use 1-29 threshold), don't hit apply
8. Analyze/Measure
9. Average distance = 8.098 Pixels (read out is always in pixels)
10. Calculate distance in microns on calibrated images

- Image/Properties, Control + Shift P (PC), Command + Shift P (Mac)
- Covert using pixel dimensions

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