Segmentation with Fiji

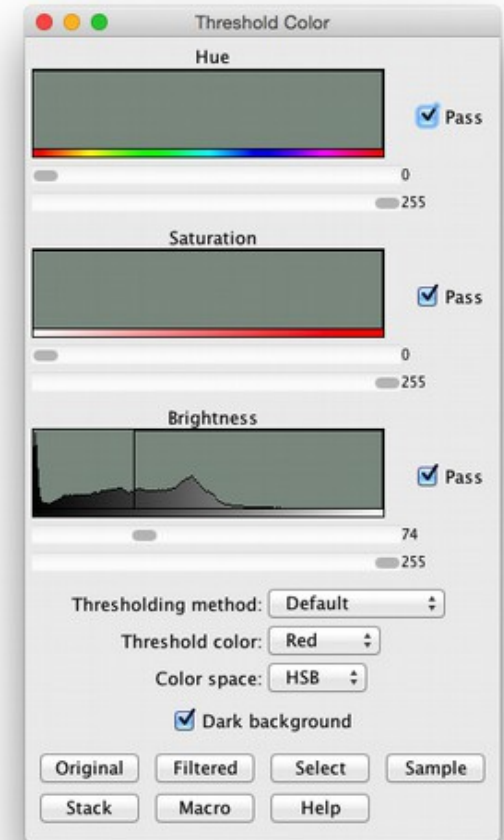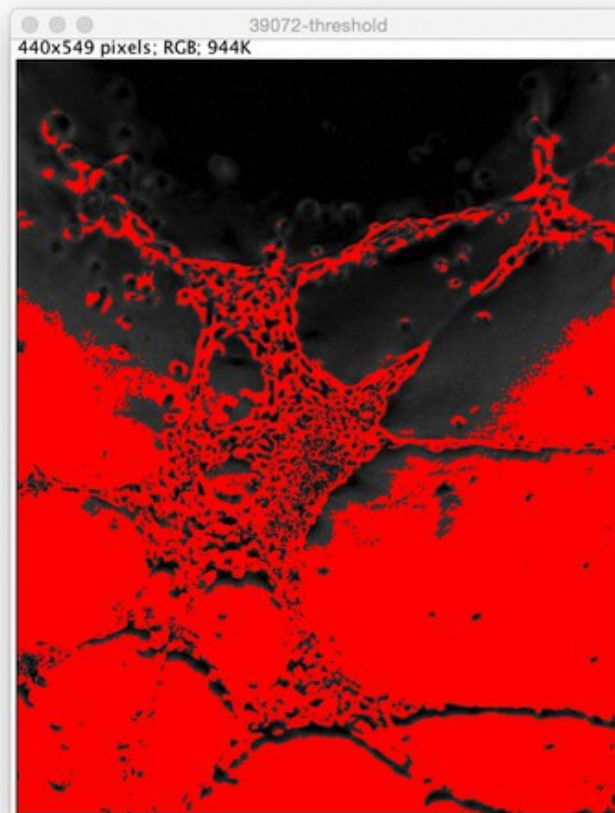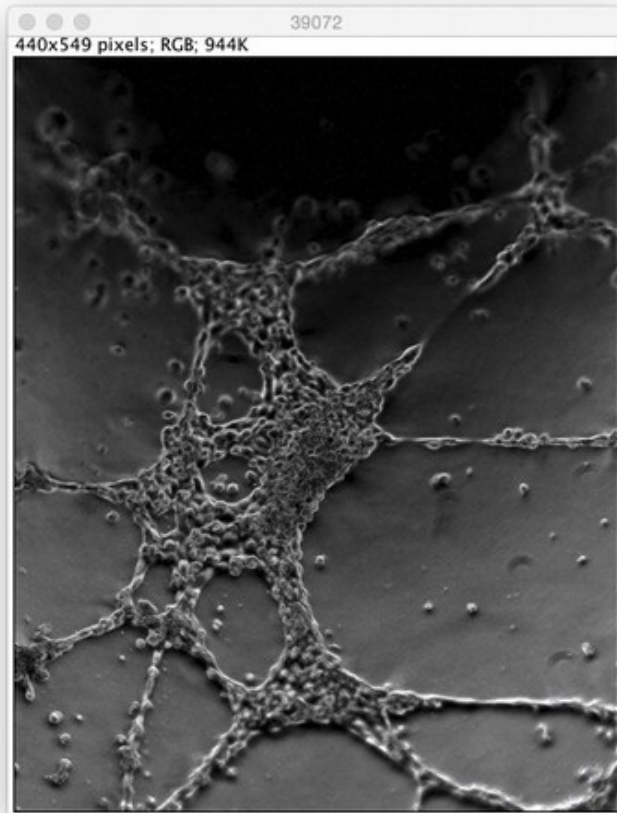http://imagej.net/Presentations

What is Segmentation?

Traditionally:
- preprocessing + thresholding

Advanced techniques:
- active contours (mostly interactive)
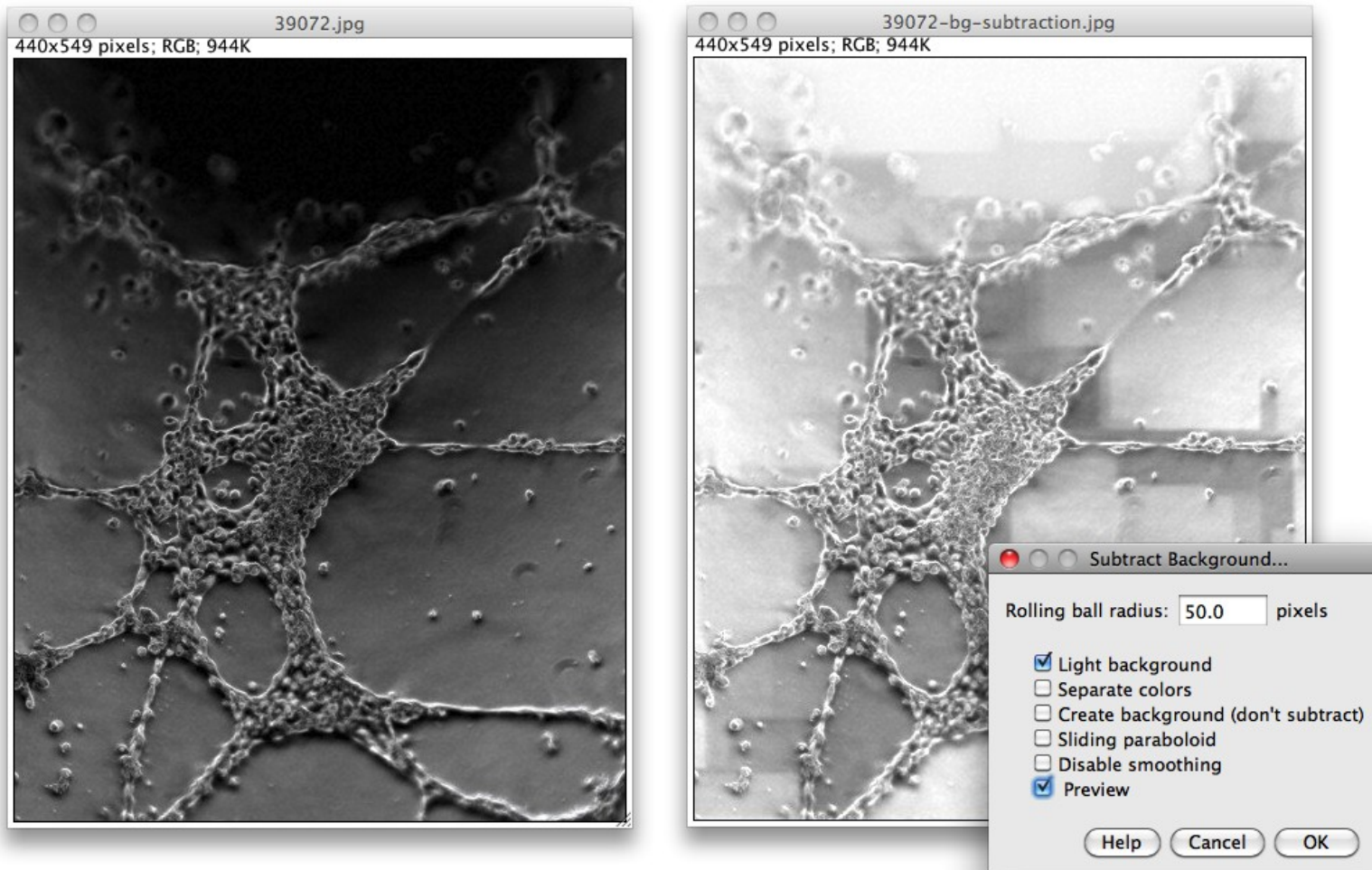- graph-based methods
- machine learning!

http://imagej.net/Segmentation

Basic Segmentation (1/2)

Apply a threshold

http://www.cellimagelibrary.org/images/39072

Basic Segmentation (2/2)

Subtract background

http://imagej.net/docs/menus/process.html#background

What's going wrong?

- Uneven illumination
- Subtract Background is too clever
- JPEG artifacts!

How can we do better?
- "wash out" details to obtain background
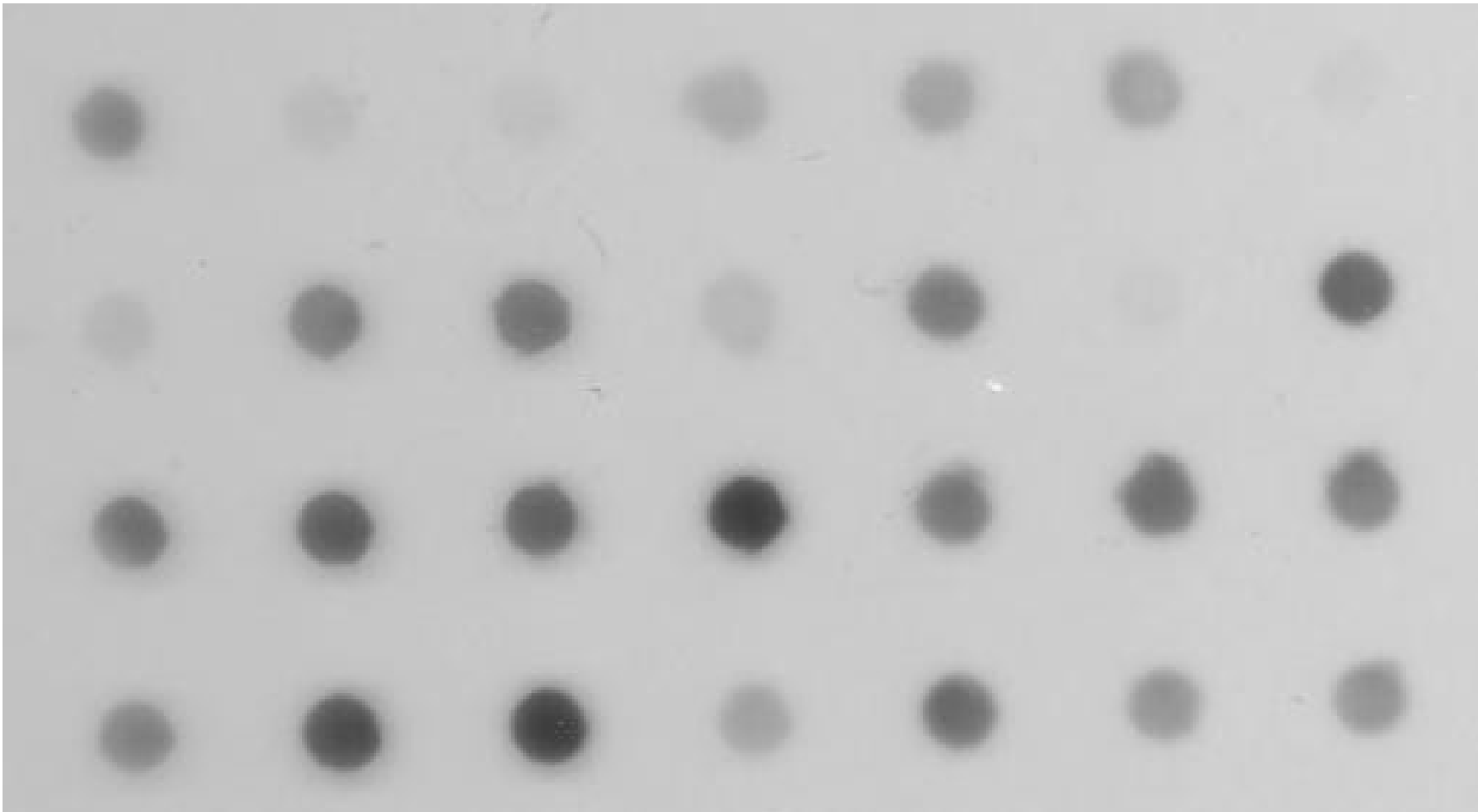- subtract background to fix illumination

Do It Yourself

- Duplicate... image
- Make 8-bit
- Apply a Gaussian blur (large radius)
- Image Calculator:
  subtract blurred image from original
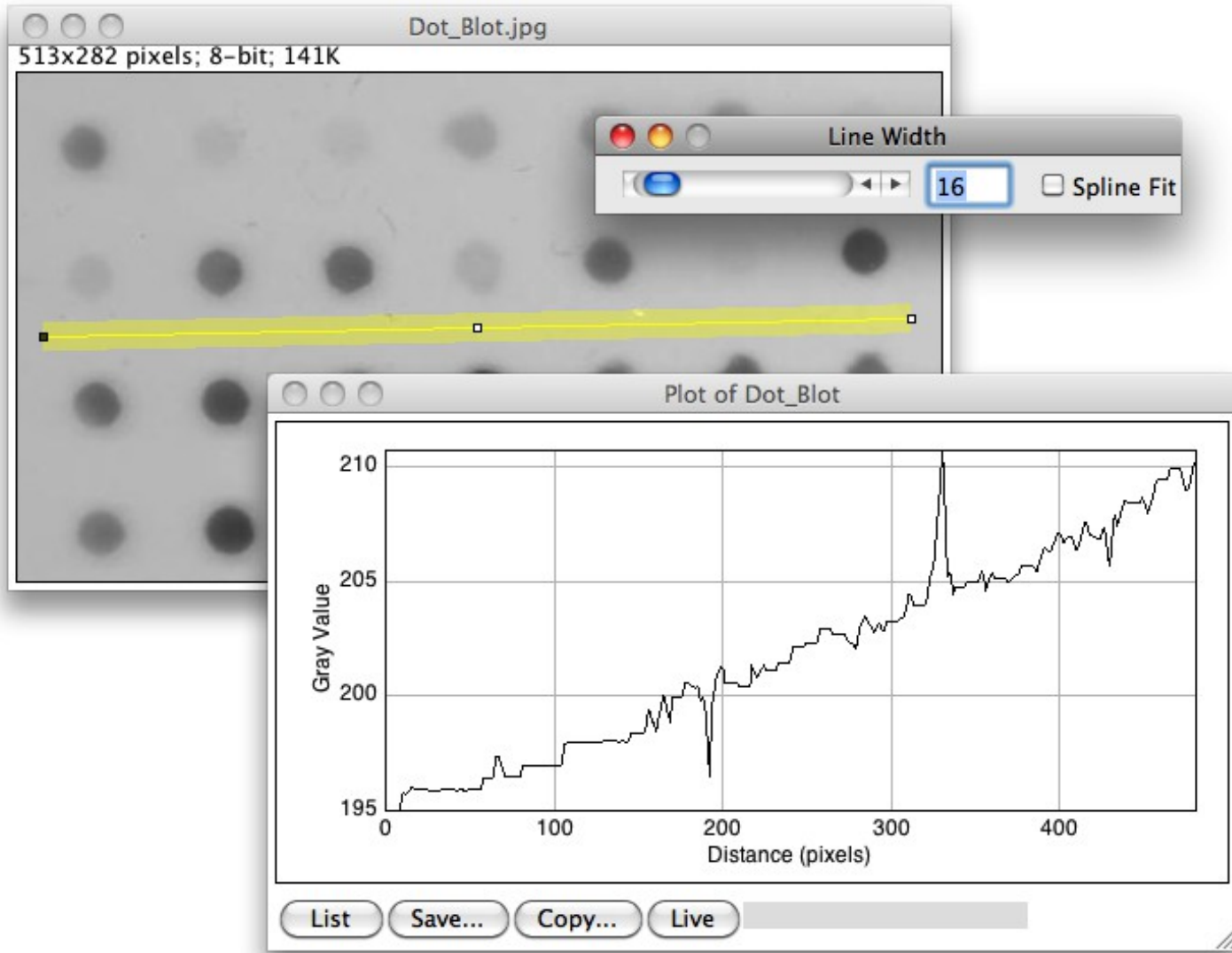- Make result 8-bit again

… that was a lot of work.
Good thing there's a *Macros* workshop!

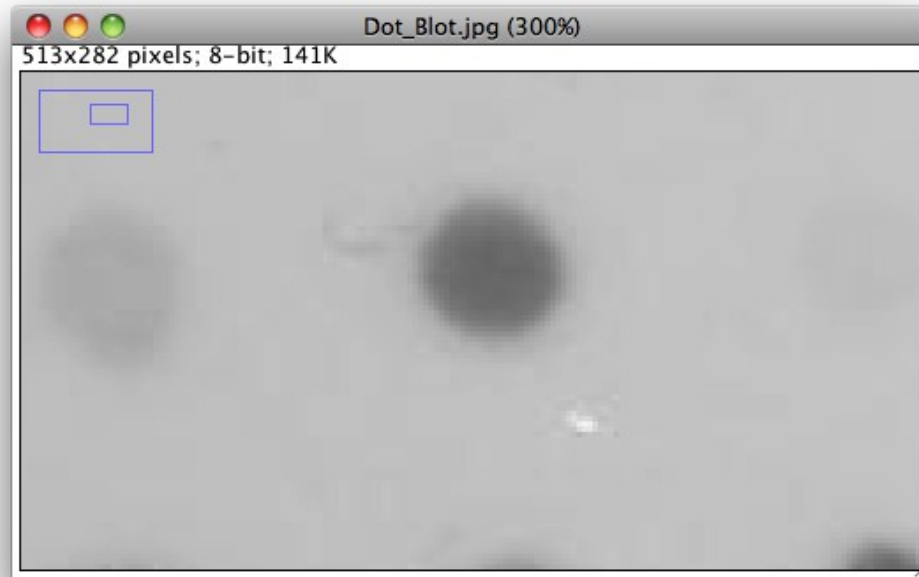# *Preprocessing*

File>Open Samples>Dot Blot (7K)

Why preprocessing?

Uneven illumination!

Why preprocessing?

Artifacts (smudges, scratches, JPEG)!

Preprocessing steps

Plan: preprocess the image to obtain a segmentation, then measure original

- Median to remove scratches
- Smooth
- Subtract background (maximum filter)

Dot Blot preprocessing

```
run("Duplicate...", "title=median");
run("Median...", "radius=7");
run("32-bit");
run("Gaussian Blur...", "radius=2");
id1 = getImageID();
run("Duplicate...", "title=max");
run("Maximum...", "radius=20");
id2 = getImageID();
imageCalculator("Subtract create 32-bit", id1, id2);
run("8-bit");
setAutoThreshold("Triangle");
```

Further Preprocessing

- Median
- Gaussian
- Bilateral filter or anisotropic diffusion
- Background subtraction
- Morphological operations with masks
- Bandpass filters

Real-world segmentation
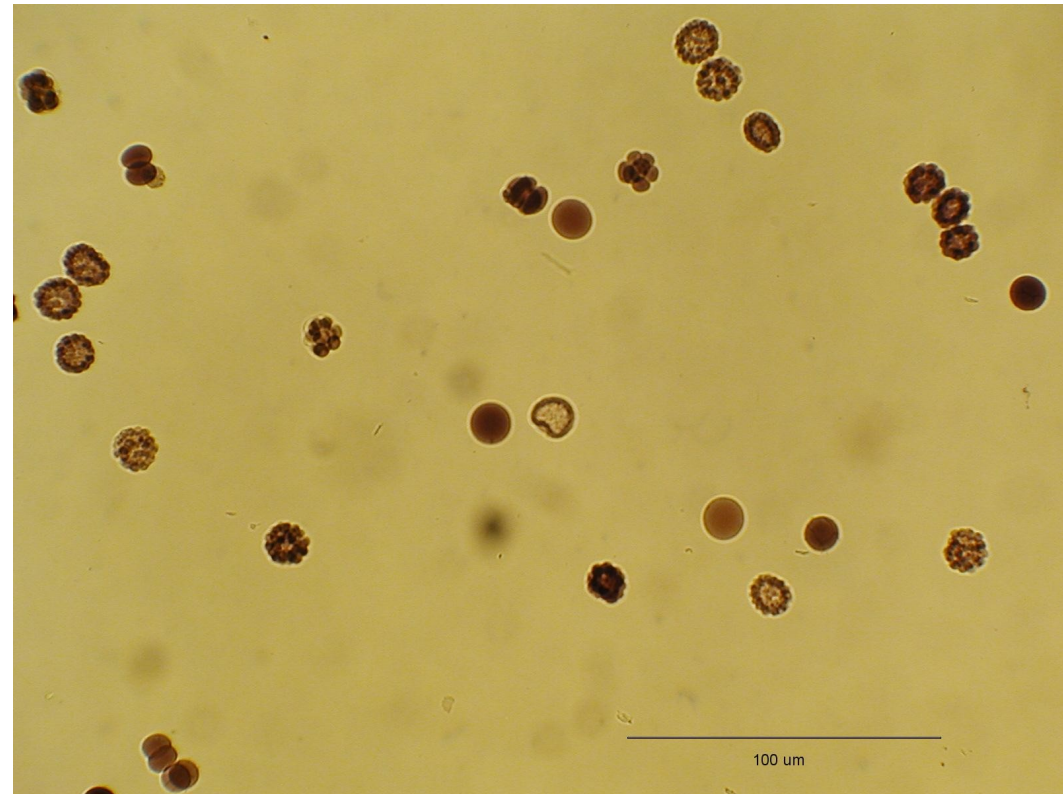
File>Open Samples>Embryos (42K)
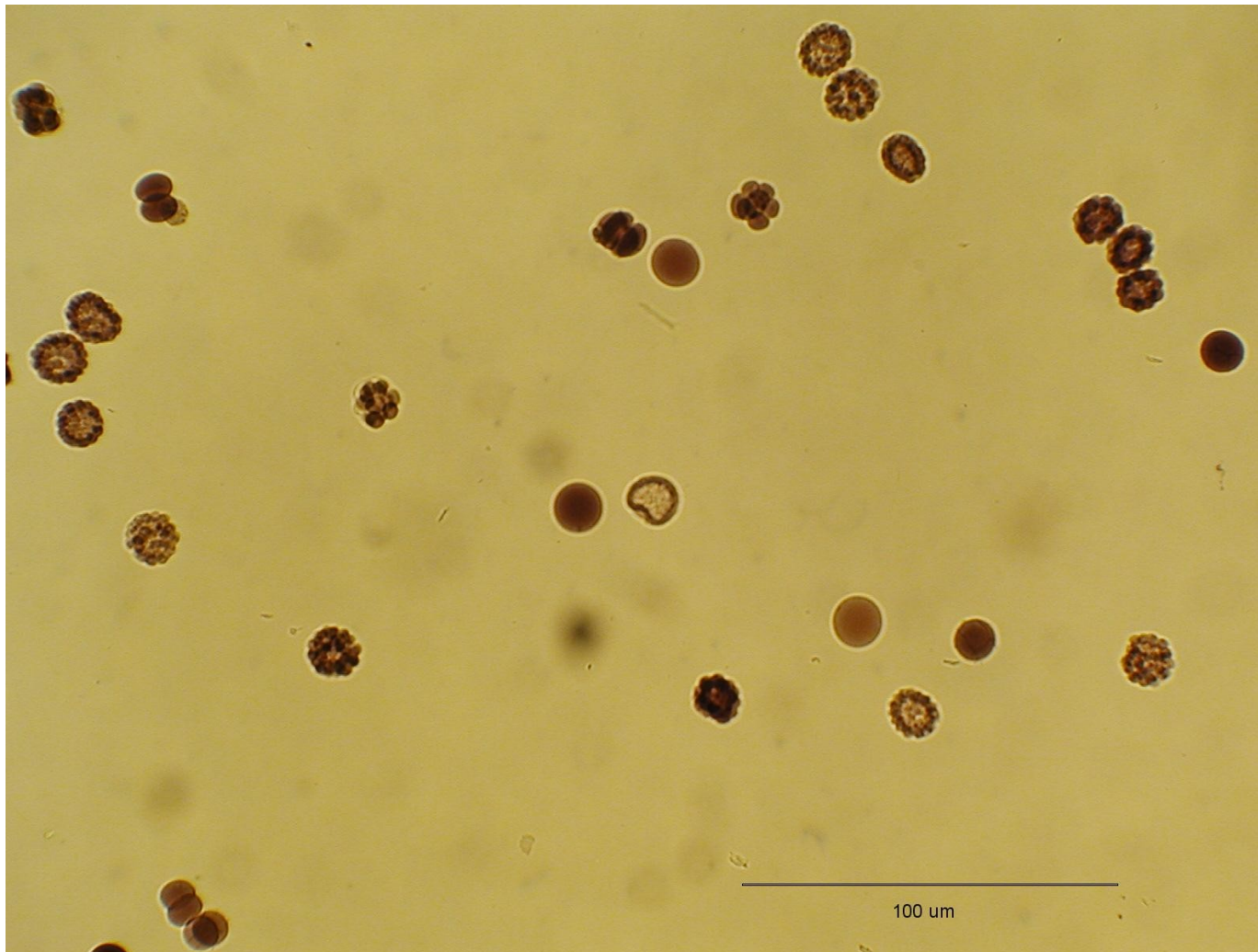
Real-world segmentation

Segment based on all channels: 8-bit
Or could use one channel: Split Channels

- Threshold
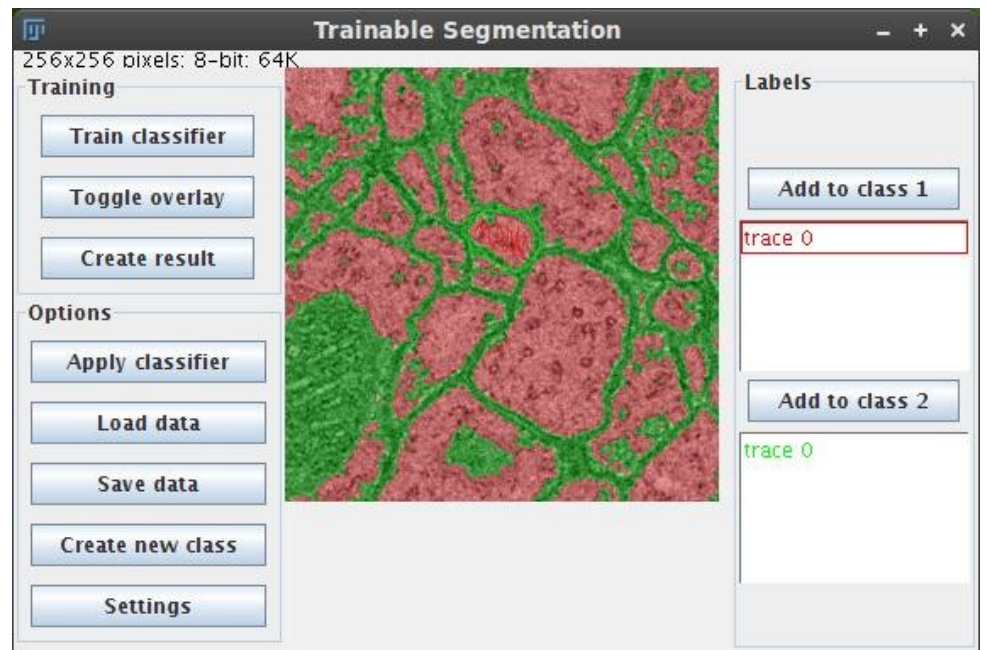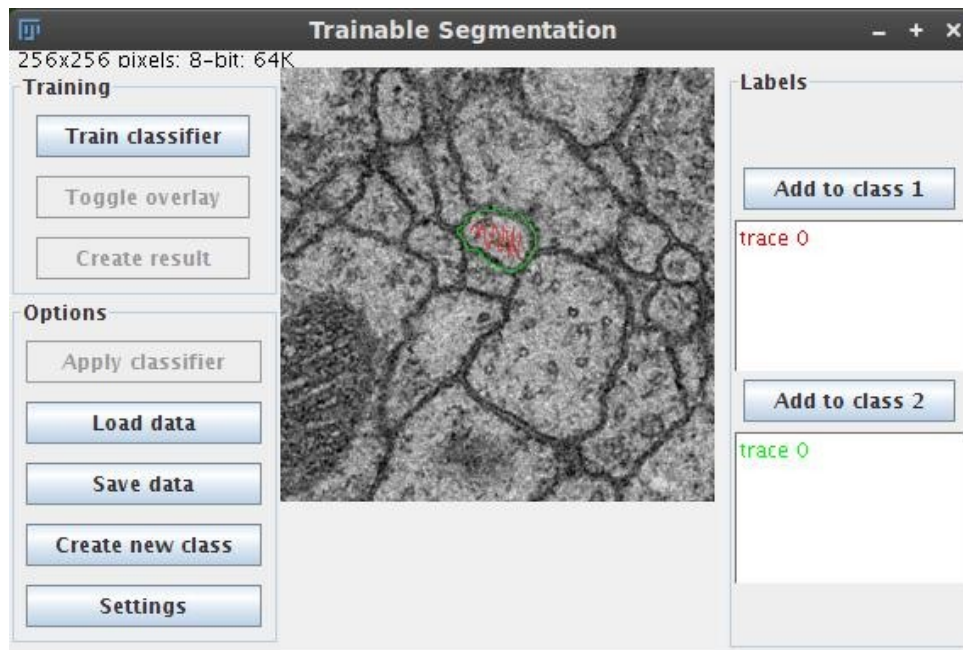- Create Mask
- Fill Holes
- Watershed
- Analyze Particles

100 um

Real-world segmentation

Challenges:

- Color
- Holes
- Touching objects
- Out-of-focus objects
- Embedded scale bar

Trainable Segmentation

Automation through Machine Learning:

http://imagej.net/Trainable_Segmentation

Further reading

Help from the community—ImageJ mailing list! ~2000 members:
http://imagej.net/Help

Segmentation overview:
http://imagej.net/Segmentation

Scripting guide:
http://imagej.net/Scripting

Additional workshops and presentations:
http://imagej.net/Presentations