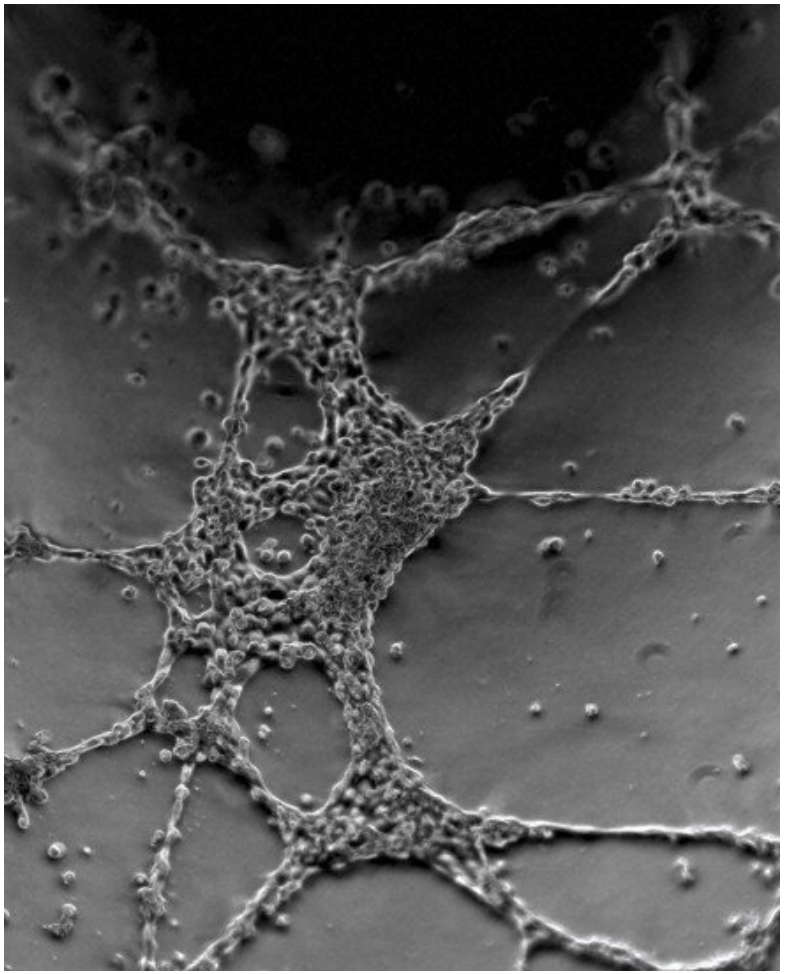
Segmentation with Fiji

http://fiji.sc/

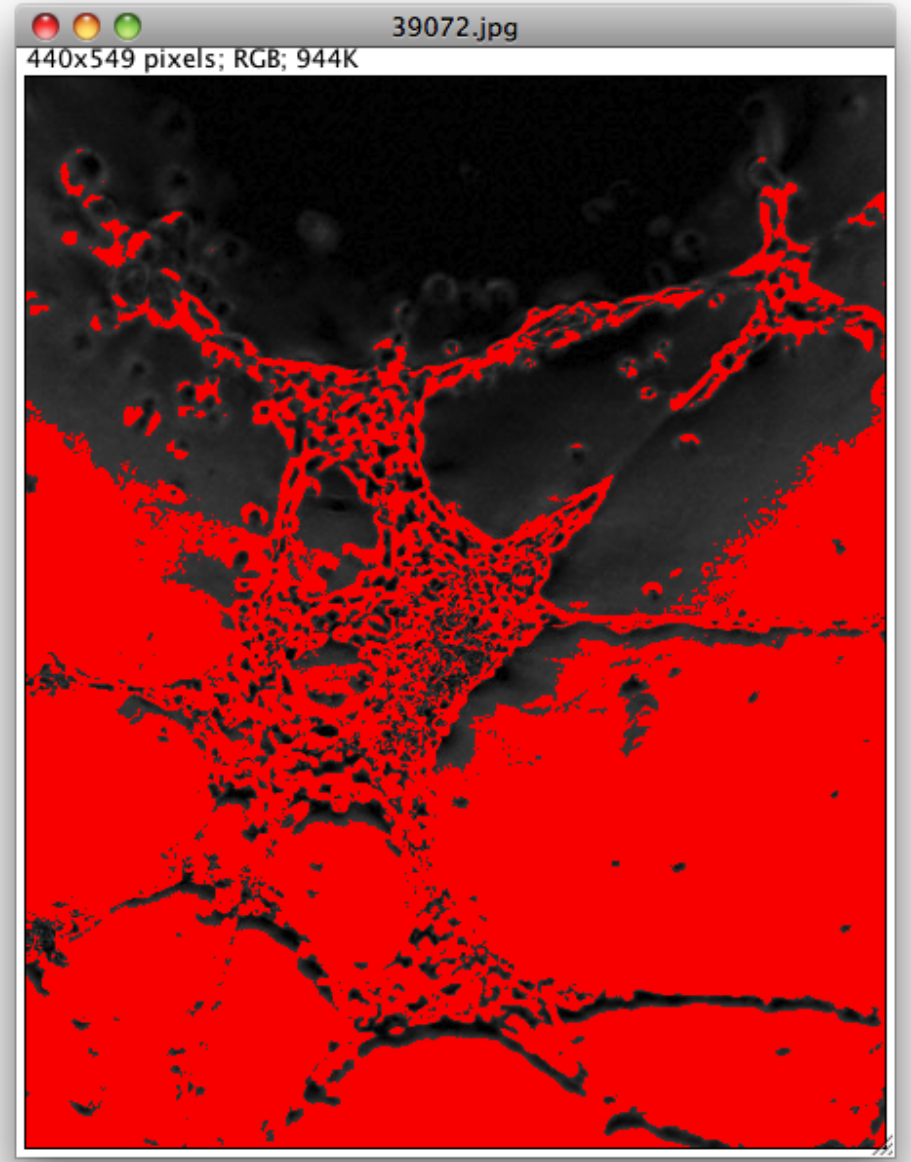
Uneven illumination



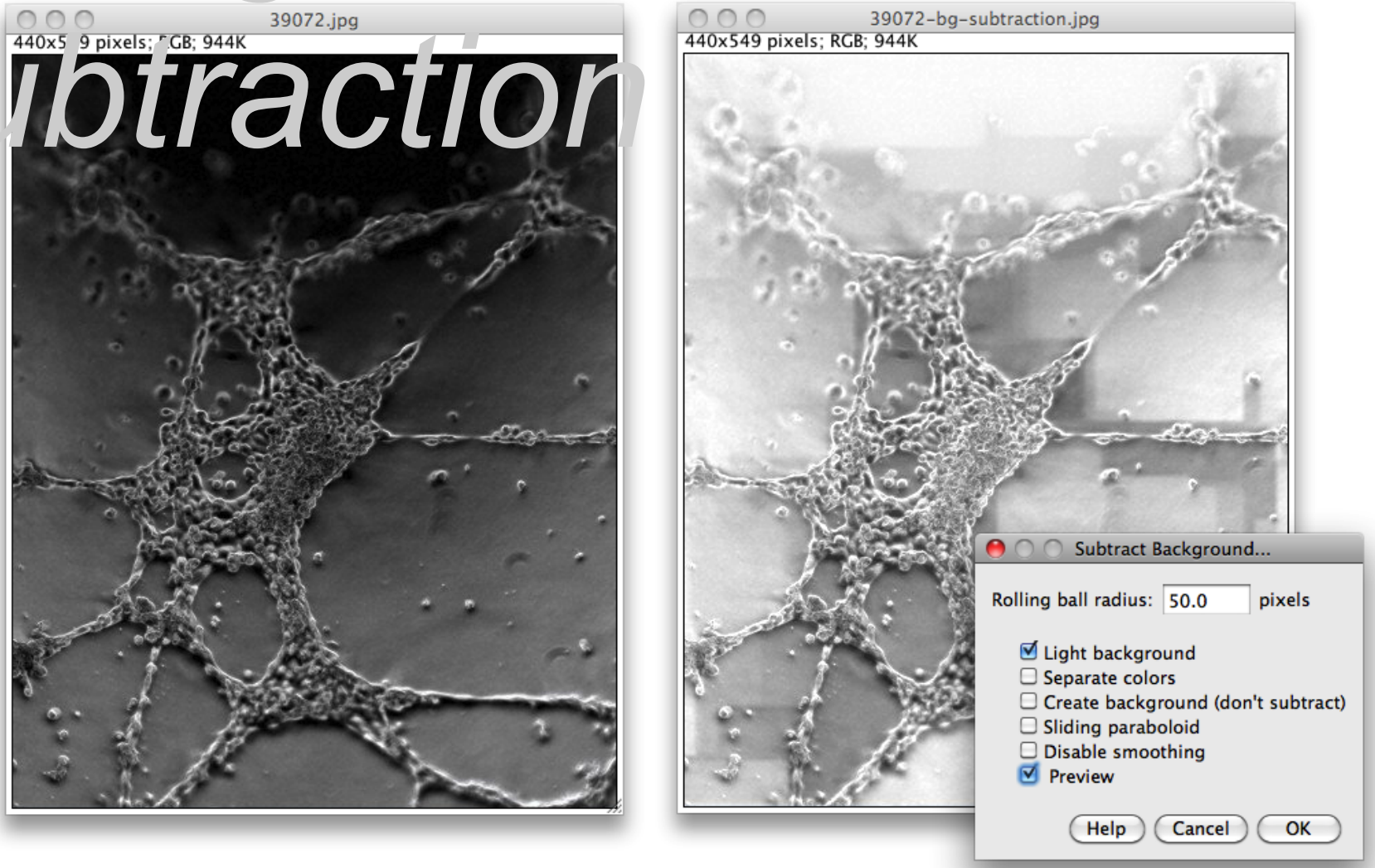
http://www.cellimagelibrary.org/images/39072

http://curtis.imagej.net/workshop/

What's the problem?



Background subtraction



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

Why did it not work?

- Subtract Background is too clever
- JPEG artifacts!

Theory is easy:

- “wash out” details to obtain background
- subtract background to compensate for uneven illumination

Do It Yourself

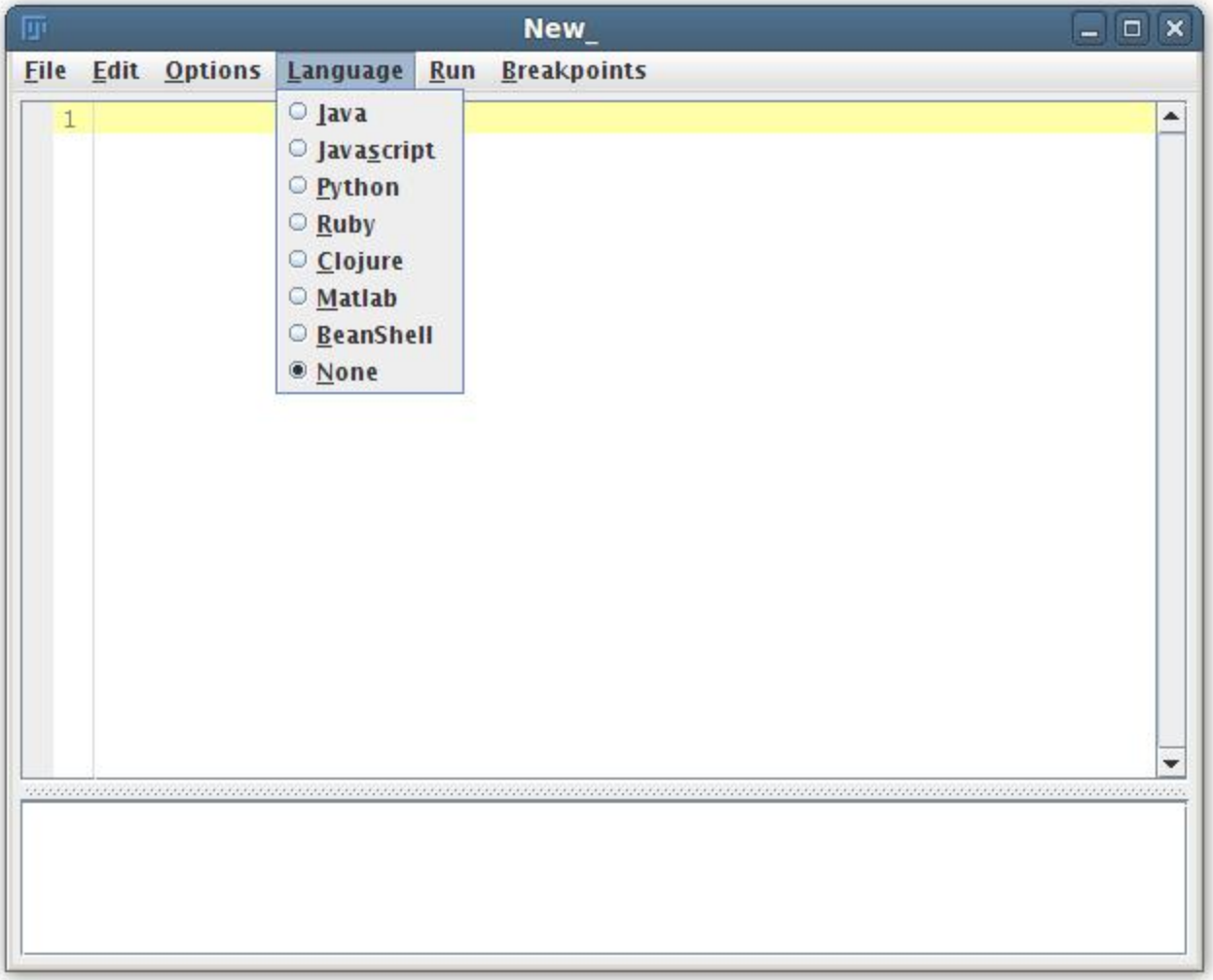
Make a macro!

- Make 8-bit
- Duplicate image
- Apply a Gaussian blur (large radius)
- Subtract blurred image (= background)
- Make 8-bit again

Improve the macro

- Batch mode
- Use Image IDs
- Store in *plugins/* (with underscores, to
  tell ImageJ to make a menu item)

Macros



- Comments
- Variables
- Functions
- String manipulation
- Conditionals
- Loops

http://imagej.net/Script_Editor

Macros: comments

```
// This is a comment trying to help you to remember
// what you meant to do here:
value = 2;

// Code can be disabled by commenting it out
// x = y * 2;
```

http://imagej.net/Macros

Macros: variables (1/2)

```
intensity = 255;

a = exp(x * sin(y)) + atan(x * y – a);

title = “Hello, World!”;

text = “title”;

text = title;
```

http://imagej.net/Macros

Macros: variables (2/2)

```
// after this, y will have the same value as x
y = x;

// now, x will be assigned a new value, but y will stay the same
x = y * y – 2 * y + 3;

// the variable is assigned after the expression is evaluated
intensity = intensity * 2;
```

http://imagej.net/Macros

Macros: functions

```
print(“Hello, world!”);

// functions can return values
number = getNumber(“Type in a number!”);

// the “run” function is the most important one
run(“Duplicate...”, “title=New”);

run(“Duplicate...”, “title=[with spaces]”);

// Try Tools>Help on Macro Functions...
// then select a function name, such as “print” and try again
```

http://imagej.net/Macros

Macros: strings

```
number = 1;

// you can concatenate strings, and strings and numbers
text = “The number is “ + number;

// why does this not work?
run(“My plugin”, “does_not_work=number”);

run(“My plugin”, “this_works=” + number);
```

http://imagej.net/Macros

Macros: conditionals

```
if (getBoolean("Is Curtis going too fast?")) {
        hint = "Tell him!";
} else {
        hint = "Try to modify the code, play with it...";
}

showMessage(hint);
```

http://imagej.net/Macros

Macros: loops

```
for (i = 1; i <= 10; i++) {
        print("Counter: " + i);
}

while (getBoolean("Are you sick of my questions yet?")) {
        print("You know, I really have all day to keep asking...");
}
```

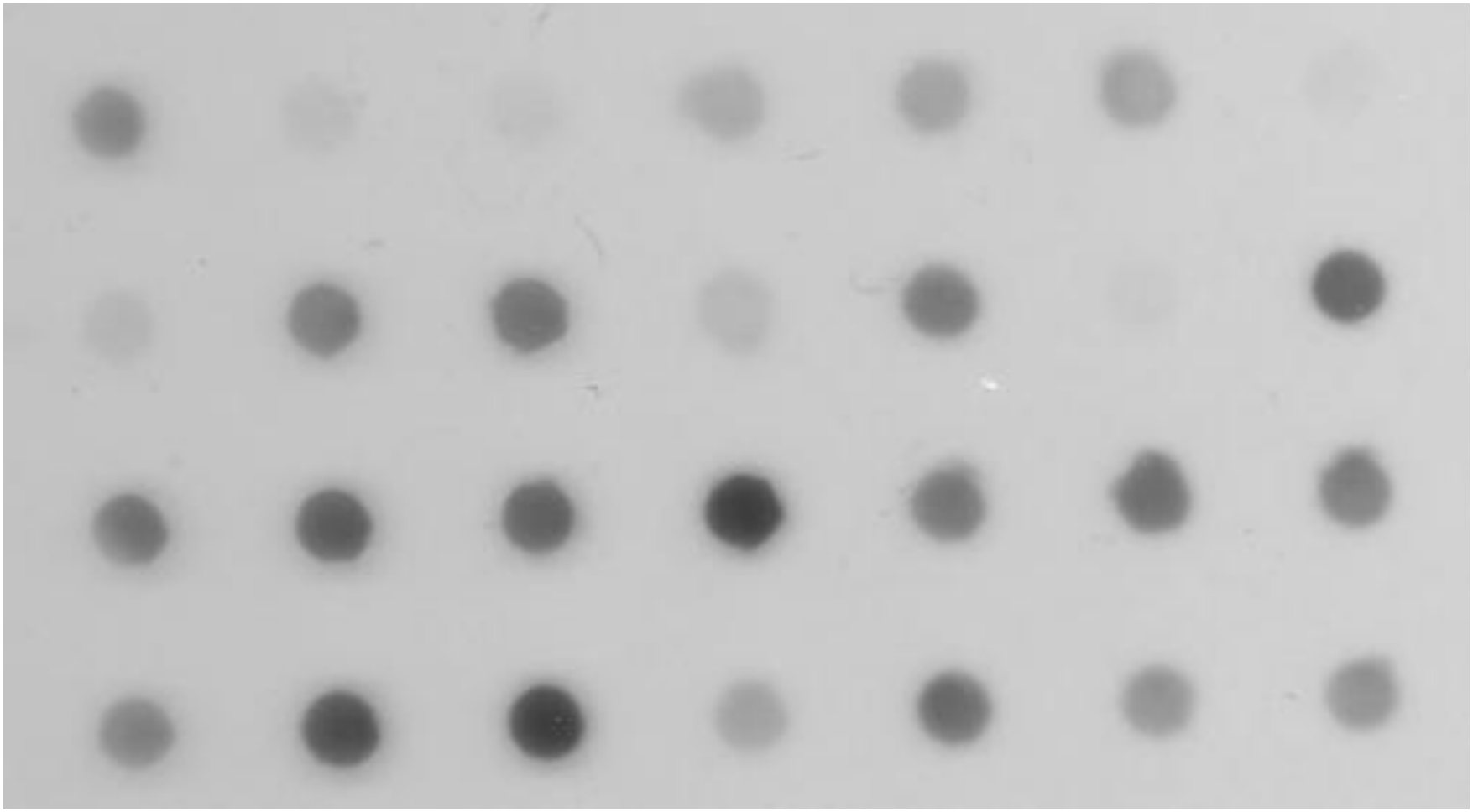
http://imagej.net/Macros

Macros: tying it together

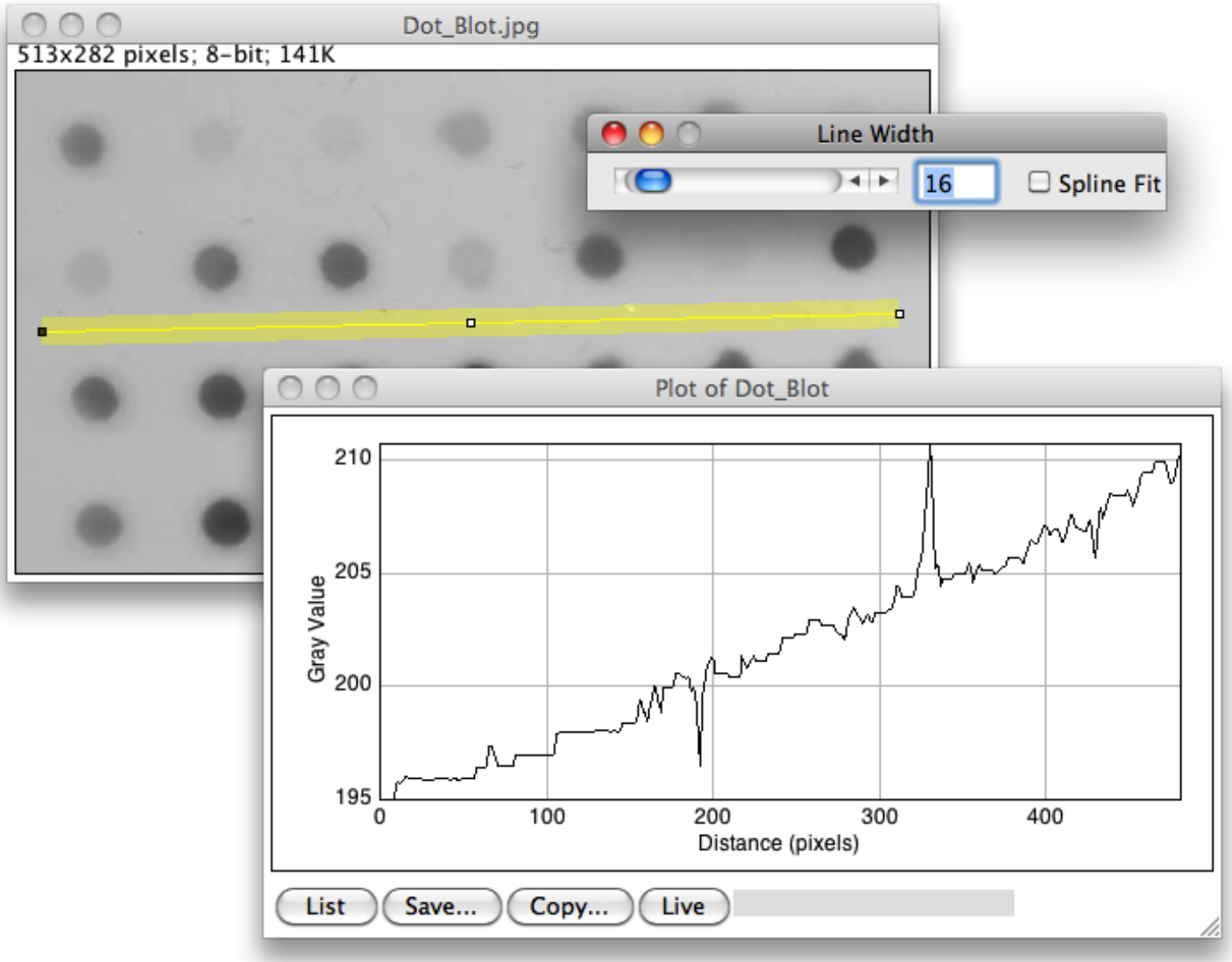
```
// this example makes a stack of blurred versions of the
// current slicewith a range of radii.

radius = getNumber("Maximal radius?");

title = "Blurred stack of " + getTitle();
run("Duplicate...", "title=[" + title + "]");
run("Select All");
run("Copy");
for (i = 1; i <= radius; i++) {
        run("Add Slice");
        run("Paste");
        run("Gaussian Blur...", "radius=" + radius);
}
```

Preprocessing

File>Open Samples>Dot Blot (7K)

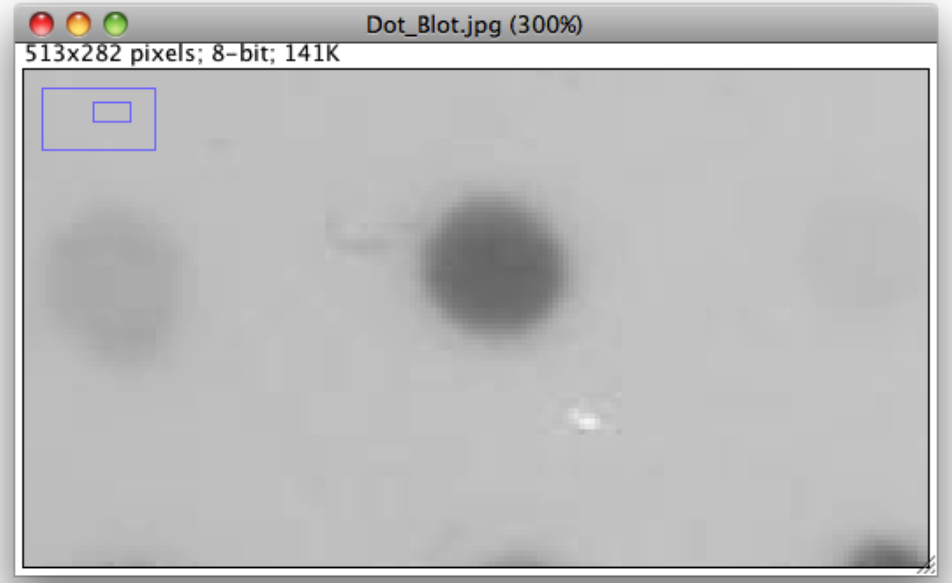


Why preprocessing?



Uneven illumination!

Why preprocessing?



Artifacts (smudges, scratches,

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");
```

Common Preprocessing

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

Segmentation

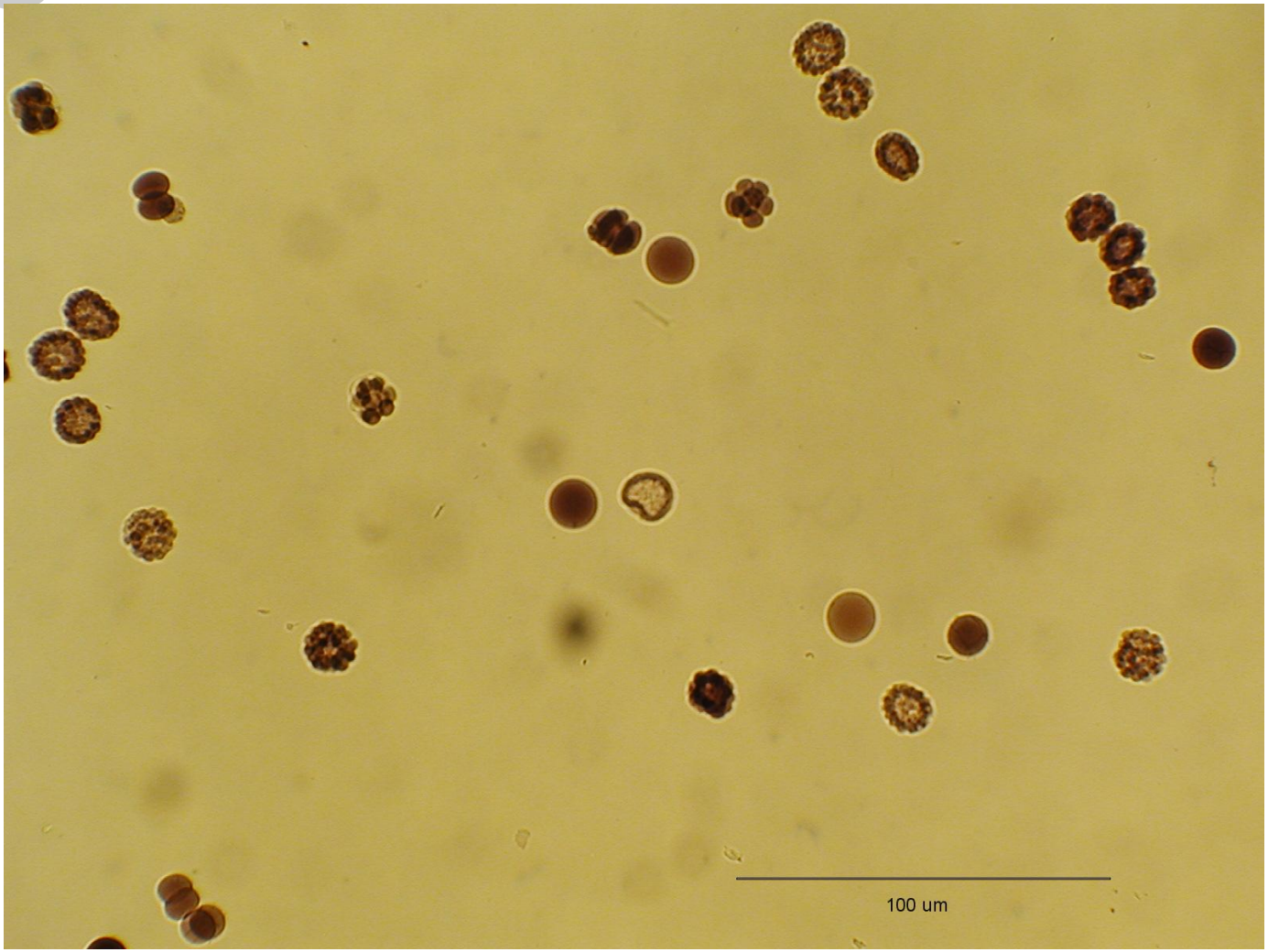
Traditionally:
Segmentation
= preprocessing + thresholding

Advanced techniques:

- Active contours (mostly interactive)
- Graph-based methods
- Machine learning!

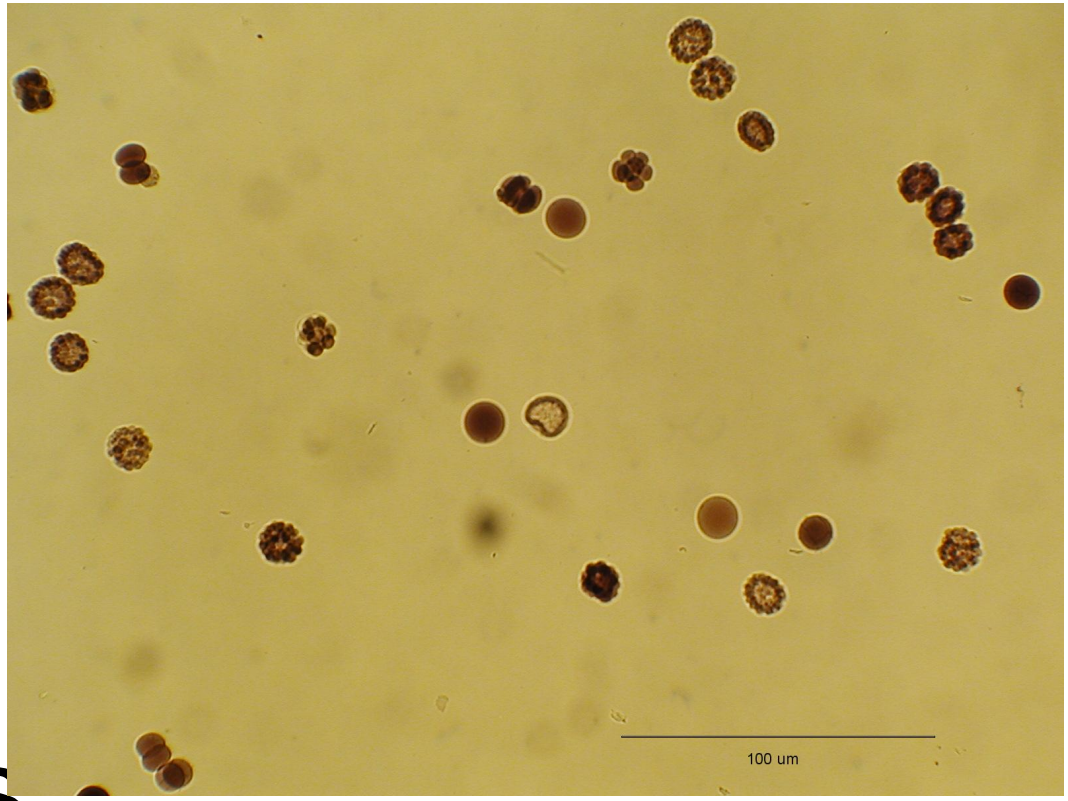
Real-world segmentation

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

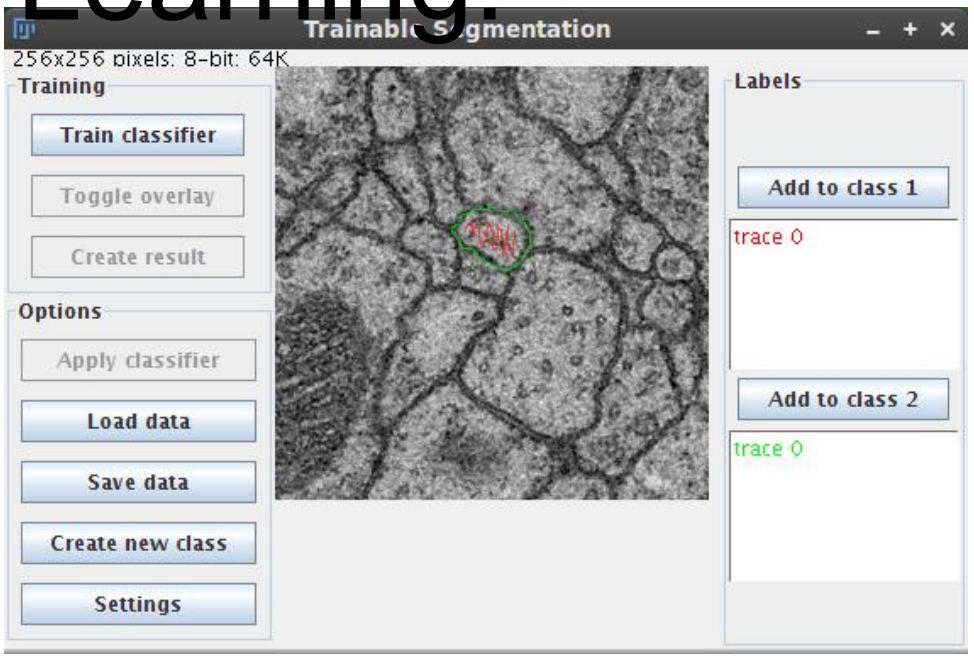
Real-world segmentation

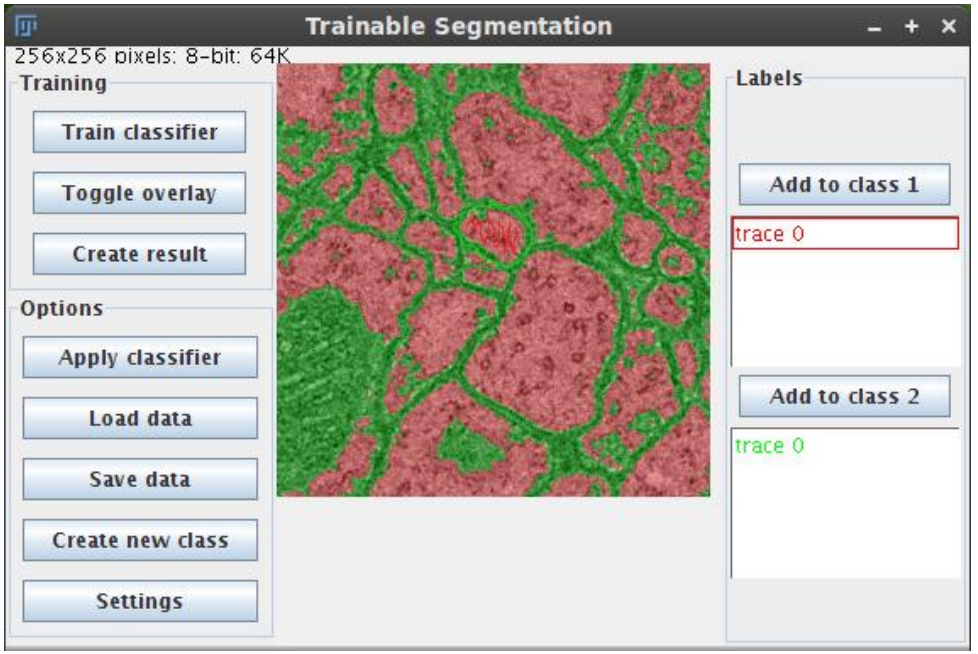
Challenges:

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

Segmentation

We will now focus on Machine Learning:





http://fiji.sc/Trainable_Segmentation

Further reading

Macro language:

http://imagej.net/Macros

Scripting guides:

http://imagej.net/Scripting

Segmentation overview:

http://imagej.net/Segmentation

Help from the community—ImageJ mailing list! ~2000 members:

http://imagej.net/Help