

## Exercise: Count Objects

Let's run a simple threshold and object counting workflow on a folder of files. We will want to threshold each image using an algorithm like Otsu and then use the `Analyze Particles` feature to count the number of particles. Then, we can run that for every file in a folder as a macro. We can use the Macro Recorder to record the workflow commands we will need for our macro.

1. Open any one of the individual images in the BBBC008 dataset mentioned in the presentation
2. Run [Plugins -> Macros -> Record]
3. Run [Image > Adjust > Threshold...] and choose Otsu, ensure `Dark Background` is ticked. You should see the nuclei marked in red in the image and the Macro Recorder has recorded the command.
4. Open [Analyze > Analyze Particles...]. Look at the checkbox options, remember this will be run *per image*, so you may want to select just Summarize. Click OK to process the image.
5. Check that the text in the Macro Recorder makes sense – it should have the Threshold command and the Analyze Particles command. Copy the commands.
6. Go back to the Script Editor. We will want to write macro code for the following:
  - a. Define a variable to store the path to the directory with the BBBC008 dataset (on macOS you can right-click on the folder while holding option to get `Copy as Pathname`. On Windows in Explorer you can use Control-L to get to the path bar and copy the path from there.
  - b. Get the list of files in the folder
  - c. Write a loop that, for each file, opens it (with the proper string) and runs your two macro commands

Remember: you can reference the Image File Handling slides and the Script Editor will give you helpful hints and autocompletions!

Note: if something isn't correct, you can use the following macro but substitute your path.

```
directory = "/Users/sobolp/Desktop/BBBC008_partial"
filelist = getFileList(directory)
for (i = 0; i < lengthOf(filelist); i++) {
    open(directory + File.separator + filelist[i]);
    setAutoThreshold("Otsu dark");
    run("Analyze Particles...", "exclude summarize");
}
```