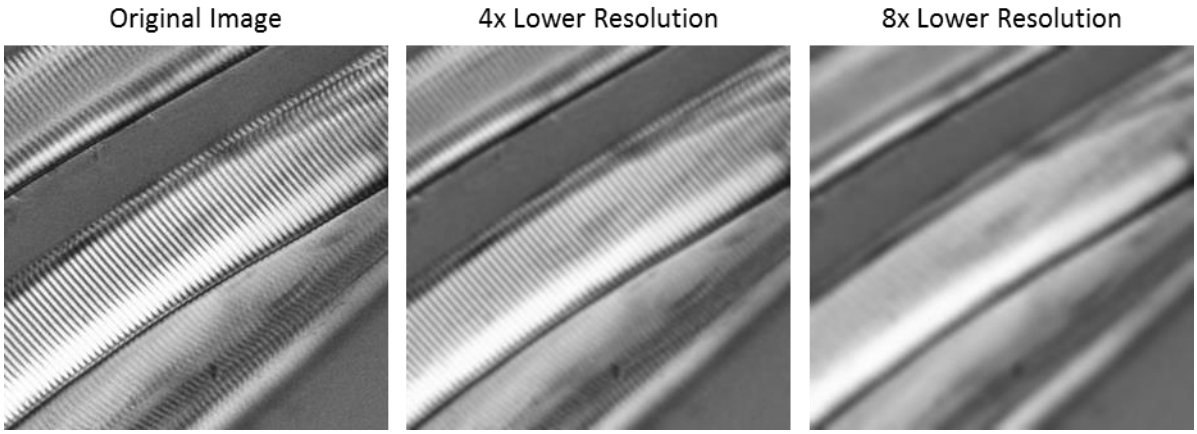
Resolution and Size Primer

Resolution

Resolution is the level of detail that you can see in an image.

We commonly encounter this concept in relation to the resolution of our TVs, smart-phones, photos, and videos, and it is exactly the same idea with the images generated by microscopes. We can think of resolution in terms of image quality, but if our ultimate goal is quantifying some aspect of our images (e.g. counting cells, measuring sizes), then we can also think about resolution as…

1. *How close two objects can be and still be distinguished, i.e. before they bleed-together into one object.* In the image series, below, you can clearly see the striations along the muscle fibers in the original image. With the resolution reduced 4x, you can still see the striations, but they are not as clear as in the original image. Reducing the resolution 8x renders the striations indistinguishable from each other; however the resolution of this image is still sufficient to distinguish the much larger individual muscle fibers.
2. *How precisely you can measure the size of a small object.* Although the striations can be resolved in both the original and 4x-lower images, the spacing between the striations can be more precisely measured from the original, high-resolution image. The striations are "fuzzier" in the 4x-lower image, which leads to uncertainty in the location of the striation edges.



If we were simply trying to distinguish the large muscle fibers, the lowest resolution image would be sufficient. However, if we needed to identify the individual striations along each fiber, we would need the highest-resolution image. How do you determine what level of resolution you need, and how do you know the resolution of your microscope system*? In digital microscopy, there are two primary settings that will determine your resolution: the optical resolution of the microscope and the digital resolution of your camera/detector.*

Optical Resolution is determined primarily by the objective numerical aperture (NA) and the wavelengths of light collected by the camera/detector. Approximate resolution limits are posted for every objective on the information sheet next to every microscope. They are just estimates because they were calculated for 500 nm light (blue/green), which may be different than the color of your sample. *Your optical resolution limit needs to smaller than the smallest object or distance you want to resolve*. For example, our best objectives will have a resolution limit of ~0.2 μm (200

nm). This means that if two objects are closer than 0.2 μm, they will appear as one object rather than two. If an object is smaller than 0.2 μm in diameter, we cannot determine its size; all we know is that it is smaller than 200 nm.

Digital resolution is determined by an image's pixel size. These sizes are also posted on the information sheet next to every microscope. They are determined by both the objective and the camera, so you will see different sizes listed for each configuration. *Your pixel size needs to be between one half and one third the size of the smallest object or distance that you want to resolve*. Most of the microscopes in the facility are configured so that the pixel size is less than half the optical resolution. The confocal microscope is different because 1) you control the pixel size and, 2) the calculation for optimal pixel size is more complicated. Recommended pixel sizes are posted next to the confocal and you will be shown during your training how to determine the best pixel sizes for your specific experiment.

> **Example**: If you want an image of two objects that are 0.5 μm apart, your optical resolution limit must be 0.5 μm or lower, and your pixel size should be 0.17-0.25 μm. If your optical resolution limit or your pixel size is larger than this, the two objects may appear as one object in your image.

Understanding and Communicating Size at the Microscale

As you can see from the previous section, it is very valuable for microscopists to understand sizes at the microscale. Explore this interactive demonstration to get a feel for how different small biological objects compare to each other in terms of size (you don't need to read the "notes" section): http://learn.genetics.utah.edu/content/cells/scale/. Consider: The resolution limit for our eyes is approximately 0.1 mm (100 μm). The optical resolution limit for standard light microscopes is 0.2 μm (200 nm). What kinds of objects cannot be resolved by your unaided eye? By a light microscope?

When you present an image, you must include a scalebar to give your audience a size reference for the objects in the image. Reporting the magnification is insufficient.

Critical Information to Record and Report: Resolution

The use and interpretation of your data by you and others depends on knowing how you acquired your images. Be sure to record the following critical parameters every time you image. If you are using a proprietary file format, most or all of these parameters may be recorded for you as metadata in the file. Many of these parameters are also posted in the information sheet next to the microscope.

- Pixel size (in μm/pixel)
- Objective numerical aperture (NA)
- The name(s) of the fluorescent or colored dyes you are using
- For fluorescence imaging: the wavelength ranges of the excitation and emission filters (or laser lines and emission detection ranges for confocal)
- For transmitted light imaging (e.g. brightfield, phase contrast, DIC): the condenser NA
- If you are acquiring z-stacks, the spacing between your slices (in μm)
- And, of course, always include a scalebar