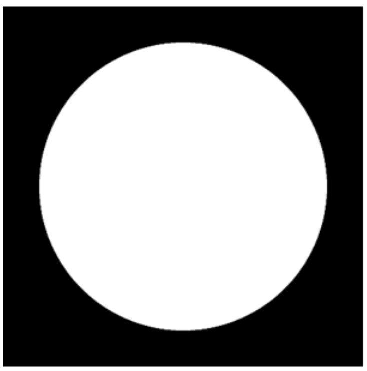
Digital Imaging Primer

What is a digital image?

Digital cameras and other light detectors, convert light intensities to electrical currents, which are then converted by a computer to numbers. Digital images are square or rectangular arrays of **pixels** (pix-el = “picture elements”), with each pixel containing a number that expresses the light intensity at that location in the image. Pixels in **monochrome** (“black and white” or “grayscale”) images only have one number each (e.g. see figure, below), while pixels in color images have three, representing the levels of red, green, and blue light. When you open a digital image, the computer takes these numbers and translates them into the intensities/colors you see on the computer screen (see figure).

Actual Object

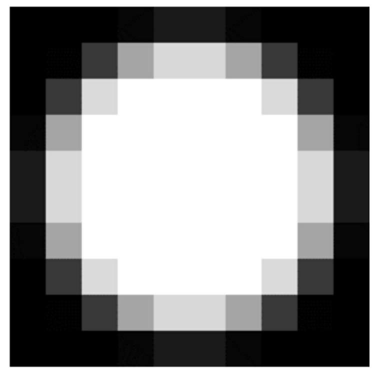


Digital Image of the Object

As it actually exists

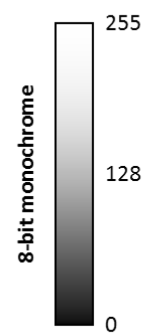
0	0	0	2	26	26	2	0	0	0
0	7	57	165	216	216	165	57	7	0
0	57	218	255	255	255	255	218	57	0
7	165	255	255	255	255	255	255	165	7
26	216	255	255	255	255	255	255	216	26
26	216	255	255	255	255	255	255	216	26
7	165	255	255	255	255	255	255	165	7
0	57	218	255	255	255	255	218	57	0
0	7	57	165	216	216	165	57	7	0
0	0	0	2	26	26	2	0	0	0

As displayed on a computer

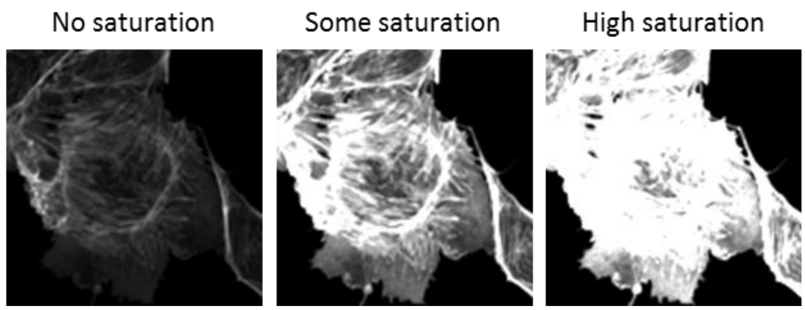


The numbers that comprise digital images are integers and are in “relative” units, meaning that the intensities are not measured on an absolute scale (e.g. lumen, candela, lux). The practical implication of this is that *you cannot directly compare intensities or colors between different images*. For example, if you see two images of cells with identical intensities, you cannot be certain that the two cells actually had the same intensity in real life. The same is true for color images: you cannot be certain that the same shades of a color in two different images are actually identical in the original specimens. This is somewhat analogous to saying, if you got an A in organic chemistry and an A in a 1-credit elective sports class, it does not necessarily mean you worked equally as hard in both classes to obtain those grades. Comparing intensities or colors between images requires control samples and very precise acquisition procedures.

Pixel values are constrained by lower and upper limits. The lowest possible value is 0, which is commonly represented as black. The highest possible value depends on the **bit-depth** of the image format. 8-bit images have $2^8 = 256$ “gray levels” and their intensity values range from 0 to 255 (one less than 256 because 0 is counted as a value). Color images are 24-bit: 8-bit each for red, green, and blue intensities (e.g. a pixel with the values 255,0,0 displays as red). Nearly all images you see in real life are 24-bit color images. Scientific cameras can acquire 12-bit (4,096 values) and 16-bit (65,536 values) monochrome images. Higher bit-depth images do not enable you to image higher intensities; rather, they give you greater precision. It is analogous to measuring your height in centimeters versus meters: I am 170 cm tall, but if we were measuring in meters only (you can only be 0, 1, 2, 3 etc. meters) I would be 2 m tall, which is clearly not as precise.



One consequence of there being an upper limit to intensity values is that, if your specimen is too bright, the camera will be unable to record accurate intensity values. All intensities above the upper limit, will simply be displayed as the highest possible value (e.g. 255 for an 8-bit image), regardless of whether that number accurately describes the intensity. This phenomenon is called **saturation** (also called clipping or over-exposure) and it causes bright regions of images to lose their detail and appear "whited-out" (see image series, above). Saturation is analogous to trying to measure the height of a group of adults using a meter-stick. Because the meter-stick isn't tall enough to reach the adults' full height, every adult will be measured as one meter tall. The solution is to change your illumination or acquisition settings to decrease the overall intensity of the specimen so that it fits within the range of the camera.



To have the best possible contrast and lowest amount of noise in your images, you want maximize your use of your camera's bit-depth without saturating. During your training, you will learn how to adjust your illumination and acquisition parameters to utilize the full range of intensity values available.

Digital Image File Formats

There are many different image file formats and they differ in several important properties. The following table shows the most common image formats you'll encounter when imaging in our facility.

Format	Preserves original data?	Retains metadata?	File size	Compatible Software
.nd2, .lif, .czi	Yes	Yes	Large	Manufacturer program; FIJI/ImageJ
TIFF	Yes	Partial or no	Large	Manufacturer program; FIJI/ImageJ
JPEG-2000	Yes	Partial or no	Medium	Manufacturer program; FIJI/ImageJ
JPEG	No	No	Small	All common programs
PNG	It depends	No	Medium	All common programs
GIF	No	No	It depends	All common programs

What file format should you use? There is a trade-off between files that preserve your images exactly as you acquired them and retain all the image's metadata (information about how it was acquired), and files that are small and easy to access. The microscope manufacturer's **proprietary format** (e.g. Nikon .nd2; Leica .lif; Zeiss .czi) preserves your images and retain extensive metadata, but are very large and can only be opened with the manufacturer's software (e.g. Nikon Elements) or the open-source program FIJI/ImageJ. On the other end of the spectrum, **JPEG** files are small and can be easily opened and imported (e.g. into Google Docs/Slides), but don't retain any metadata and use a "lossy" compression that changes your pixel values. *A good strategy is to save your original images in the relevant proprietary format and then, when necessary, export a copy of the image in a different format.* For example, you may save a copy as a PNG or JPEG to import into a PowerPoint presentation or as a TIFF to create a figure for a manuscript. Never save your original images in JPEG or PNG format! There are other file formats and reasons for using them that will be discussed, if applicable, during your training.

Critical Information to Record and Report: Digital Imaging

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.

- Image bit-depth
- Camera (or other light detector) name
- Objective magnification
- Important illumination and camera/detector settings (varies by instrument; will discuss during your training)