Chapter 1: What is an image?

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What is an image?

An image is a static snapshot of a scene. Let us start with an analog image and work through understating a digital image. An example of an analog image is a photograph taken with an old fashioned camera on a physical photosensitive film. If we were to take a large magnifying glass and zoom in on a really small area, the colors on the image will change in a very 'smooth' manner without any discontinuities. Such an image is an analog image, which is in essence a 'perfect' representation of the captured scene where every possible detail is captured.

Our computers are extremely good at storing an manipulating massive amounts of numerical values. That is they store discrete digital values, and hence impossible for them to store numerical values for infinitesimally small intervals when moving along an image. Our computers store a 'digitized' version of an image. An image is digitized by diving an analog image into grids. Each grid represents a pixel, and each pixel stores the average value of the light falling in that square, that is each pixel stores one value. The smaller the grid the smaller the area of the image capture by the grid, and hence more grids are required to cover the entire image. Making the grids smaller increased the resolution of the image, however it comes at the cost of storing more values per image (and so increasing the image size).

Obviously, all digital cameras and microscopes do not capture an analog image and convert them to a digital image, but rater they capture a digital image directly. The most common methods used for capturing digital images in a microscope are CMOS-CCD such as used in most wide-field microscopes and PMT such as used in most confocal microscopes. The details of how these senors work are irrelevant here, and we directly obtain a final digital image from them.

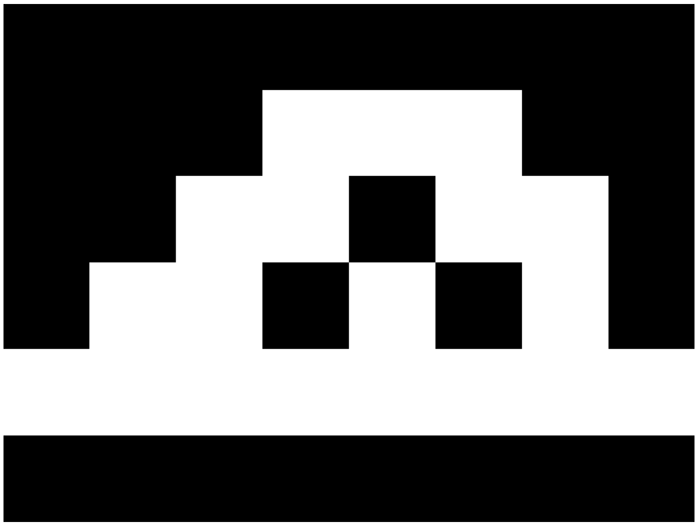
Now that we have divided an image into a fixed number of grids and have the average intensity at each grid, how are these values actually stored in a computer and how can we manipulate them? We will use make extensive use of two python packages,`numpy` and `scikit-image`, for our image analysis.

An image is a matrix

Binary image

As we have seen an image is a grid of pixels. A grid is most easily represented as matrix. The dimensions of the matrix corresponding to the size of an image. Each element of the matrix corresponds to a pixel. A two dimensional matrix of size 6 * 8 would represent an image that is 6 pixels in height and 8 pixels in width, and the intensity is stored at each pixel. Images in `ski-image` are represented as `numpy` arrays:

```python
# Load the required packages
import skimage as ski
import numpy as np
import matplotlib.pyplot as plt

# A 2D array
img = np.array([[0, 0, 0, 0, 0, 0, 0, 0],
                [0, 0, 0, 1, 1, 1, 0, 0],
                [0, 0, 1, 1, 0, 1, 1, 0],
                [0, 1, 1, 0, 1, 0, 1, 0],
                [1, 1, 1, 1, 1, 1, 1, 1],
                [0, 0, 0, 0, 0, 0, 0, 0]])

# An we can view an image just like a plot
fig, ax = plt.subplots()
ax.imshow(img, cmap = 'gray')
plt.axis('off')
```



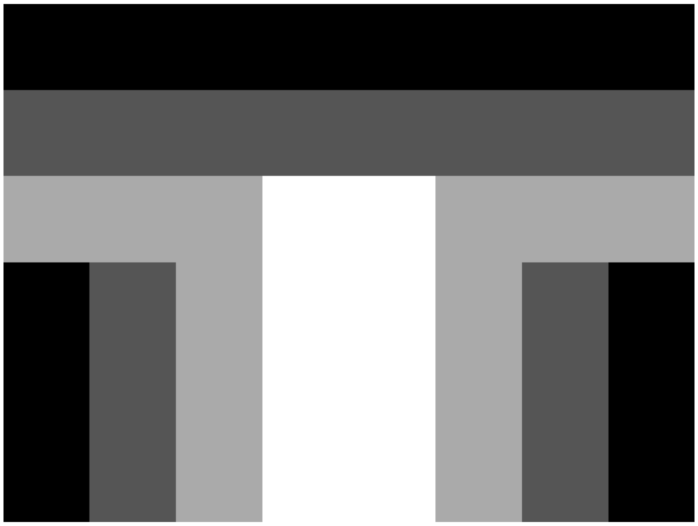
Here, all the pixels have a value of a `0` or a `1` and it represent just two colors. In a binary image, a `0` typically corresponds to a black pixel and a `1` to a white pixel (however, these are just arbitrary pseudo-colors for visualization).

Grayscale image

A grayscale image is similarly a matrix but each pixel contains a larger range of values. The minimum value corresponds to a black pixel, the maximum value to a white pixel, a the values in between represent the distinct shades of gray. Therefore, the larger the range of values, the more shades of gray can be represented.

In the simplest case, let us consider a 2-bit image, where the smallest value at a pixel is 0 and the largest value is $2^2 - 1 = 3$. Here, 0 represents black and 3 represents white, and 1 and 2 represent shades of gray in between.

```
# A 2D image with 4 distinct values
img = np.array([[0, 0, 0, 0, 0, 0, 0, 0],
                [1, 1, 1, 1, 1, 1, 1, 1],
                [2, 2, 2, 3, 3, 2, 2, 2],
                [0, 1, 2, 3, 3, 2, 1, 0],
                [0, 1, 2, 3, 3, 2, 1, 0],
                [0, 1, 2, 3, 3, 2, 1, 0]])

# plot the image
fig, ax = plt.subplots()
ax.imshow(img, cmap = 'gray')
plt.axis('off')
```

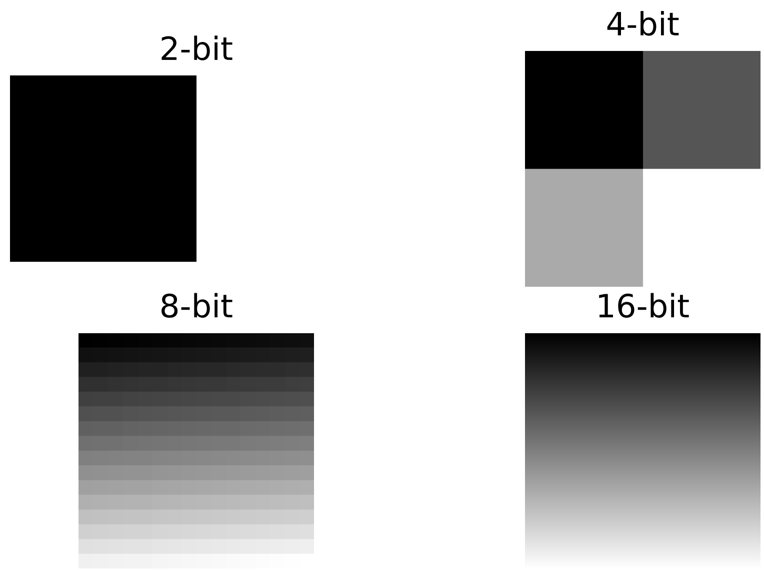


In an 8-bit image we can have $2^8 = 256$ distinct values, with 0 being black, 255 being white, and 254 values of gray in between. Similarly a 16-bit image can have $2^{16} = 65536$ distinct values. Therefore, as the bit-depth of an image increases we can it can represent an image with more shades of gray.

The figure below shows 2, 4, 8, and 16 bit images, with `0` at the top right and the maximum value at the bottom left, and each pixel increments by `1`. Notice how the increment in intensity between adjacent pixels get smaller as the bit-depth of the image increases.

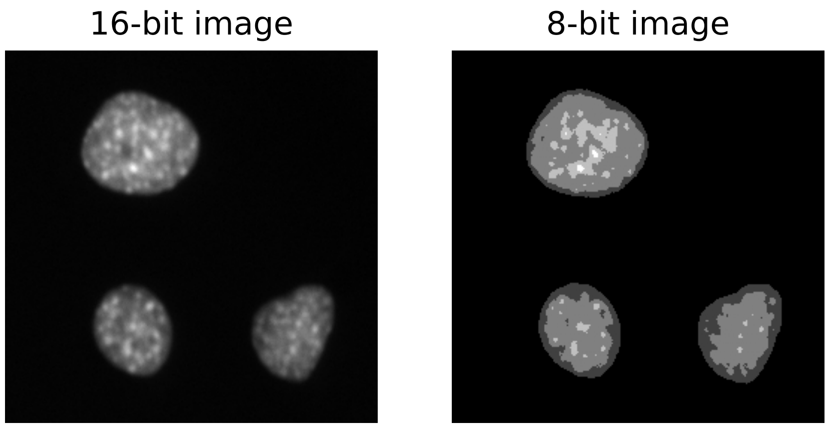
```
# A 1x2 2-bit image
img_2bit = np.arange(0, 2).reshape(1,2)
# A 2*2 4-bit image
img_4bit = np.arange(0, 4).reshape(2, 2)
# A 16*16 8-bit image
img_8bit = np.arange(0, 256).reshape(16, 16)
# A 256*256 16-bit image
img_16bit = np.arange(0, 65536).reshape(256, 256)

# plot the images
fig, ax = plt.subplots(2, 2)
ax[0, 0].imshow(img_2bit, cmap = 'gray')
ax[0, 0].set_title('2-bit')
ax[0, 0].set_axis_off()
ax[0, 1].imshow(img_4bit, cmap = 'gray')
ax[0, 1].set_title('4-bit')
ax[0, 1].set_axis_off()
ax[1, 0].imshow(img_8bit, cmap = 'gray')
ax[1, 0].set_title('8-bit')
ax[1, 0].set_axis_off()
ax[1, 1].imshow(img_16bit, cmap = 'gray')
```

```
ax[1, 1].set_title('16-bit')
ax[1, 1].set_axis_off()
```



16-bit images are the most typical in microscopy since it can capture a large amount of detail per pixel. For example, the figure below show the same nuclear image in 16 bit and in 8 bit. Notice how much more detail can be seen in the 16 bit image than in the 8-bit image.

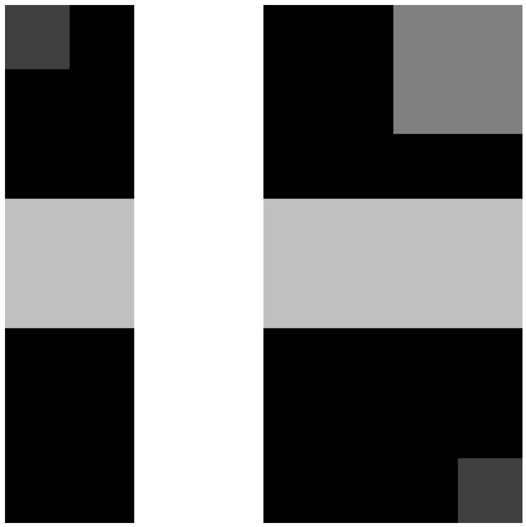
```
# Read in an image
img_16bit = ski.io.imread("data/F01_202w2.TIF")[150:400, 1100:1350]
img_8bit = ski.util.img_as_ubyte(img_16bit)

fig, ax = plt.subplots(1, 2)
ax[0].imshow(img_16bit, cmap = 'gray')
ax[0].set_title('16-bit image')
ax[1].imshow(img_8bit, cmap = 'gray')
ax[1].set_title('8-bit image')
for a in ax:
    a.set_axis_off()
```



Indexing and manipulating images

Since an image is represented as a matrix, we can index and manipulate an image just like a matrix. The image coordinate `[0, 0]` is at the top left of the image in `numpy`. As the x-coordinate increases we go down the image, and as the y-coordinate increases we go the right of the image. A few examples to get started with image indexing are shown below.

```
# Create an 8x8 image with all 0s
img = np.zeros((8, 8))

# Index a specific pixel by specifying the both the x and y coordinates
# top left pixel
img[0, 0] = 1
# bottom right pixel
img[7, 7] = 1

# Index a range of pixels by specifying the start and stop coordinates
# for both x and y coordinates. The coordinates are specified in
# format `start:end-1`.
# Set the top right of the image
img[0:2, 6:8] = 2

# We can access all the columns by specifying ':' as the column index
img[3:5, :] = 3
# We can access all the rows by specifying ':' as the row index
img[:, 2:4] = 4

fig, ax = plt.subplots()
ax.imshow(img, cmap = 'gray', vmax = 4)
plt.axis('off')
```

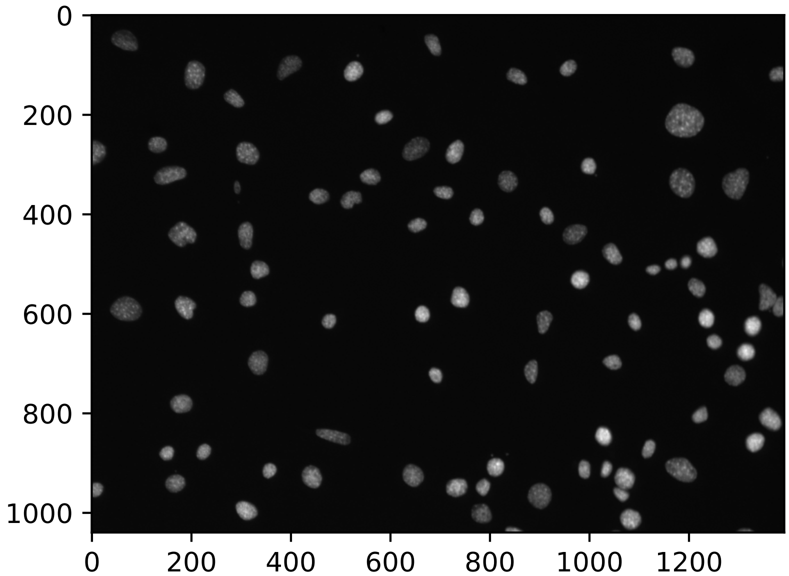


Reading and writing images

Now that we know how to manipulate toy images, let us read in a real image. We can read an image using the function `io.imread` to read an image from file to a numpy ndarray, all we need is a path to the image. Once we have the image, we can view and manipulate it in the same way. To write an image we can use the function `io.imsave`, we need the image to save and the file path.

```
# Read in an image
img = ski.io.imread("data/F01_202w2.TIF")

# we can check the size of the image and the data type
print(img.shape)
print(img.dtype)

# We can similarly view the image using matplotlib
fig, ax = plt.subplots()
ax.imshow(img, cmap = 'gray')

# Manipulate the image
img[200:500, 300:400] = 0
img[:, 8] = 0

# Write the manipulated image to file.
ski.io.imsave('data/out.tif', img, check_contrast = False)
```

```
(1040, 1392)
uint16
```



A note on image formats

Image formats determine how the pixel and intensity information of an image are stored in a computer. Broadly there are two classes of file types: (1) lossy compression formats such as JPEG, PNG, etc. (2) Uncompressed (or lossless compressed) formats such as TIFF (or, shortly, TIF) and most microscopy image formats. Lossy compressed files are smaller but they lose information in order to achieve the smaller file size. Never use a lossy compressed files for microscopy or image analyses. TIFF file formats are very widely for image analysis, and it is highly recommended. Most proprietary file formats that is output from microscopes can be converted to TIFFs.