


# Image Handling

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## OVERVIEW

### Questions

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- How to read and process images in Python?
  - How is an image mask created?
  - What are colour channels in images?
  - How do you deal with big images?
- 

### Objectives

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- Understanding 2-dimensional greyscale images
- Learning image masking
- 2-dimensional colour images and colour channels
- Decreasing memory load when dealing with images

Images: Gray Image



Images: Colour Image



Images: Masking



## PREREQUISITES

- NumPy arrays
- Plots and subplots with matplotlib

## PRACTICE EXERCISES

This lesson has no explicit practice exercises. At each step, use images of your own choice to practice. There are many image file formats, different colour schemes etc for which you can try to find similar or analogous solutions.

## Challenge

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### Reading and Processing Images

In biology, we often deal with images; for example, microscopy and different medical imaging modalities often output images and image data for analysis. In many cases, we wish to extract some quantitative information from these images. The focus of this lesson is to read and process images in Python. This will include:

- Working with 2-dimensional greyscale images
- Creating and applying binary image masks
- Working with 2-dimensional colour images, and interpreting colour channels
- Decreasing the memory for further processing by reducing resolution or patching
- Working with 3-dimensional images

### Image Example

The example in [Figure 1](#) is an image from the cell [image library](#) with the following description:

“Midsaggital section of rat cerebellum, captured using confocal imaging. Section shows inositol trisphosphate receptor (IP3R) labelled in green, DNA in blue, and synaptophysin in magenta. Honorable Mention, 2010 Olympus BioScapes Digital Imaging Competition®.”

Given this information, we might want to determine the relative amounts of IP3R, DNA and synaptophysin in this image. This tutorial will guide you through some of the steps to get you started with processing images of all types, using Python. You can then refer back to this image analysis example, and perform some analyses of your own.


 Figure 1: Example image, rat cerebellum

Figure 1: Example image, rat cerebellum

## Work-Through Example

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## Reading and Plotting a 2-dimensional Image

Firstly, we must read an image in. To demonstrate this we can use the example of a histological slice through an axon bundle. We use Matplotlib's image module, from which we import the function `imread`, used to store the image in a variable that we will name `img`. The function `imread` can interpret many different image formats, including .jpg, .png and .tif images.

PYTHON < >

```
from matplotlib.image import imread

img = imread('fig/axon_slice.jpg')
```

OUTPUT < >

```
PIL.UnidentifiedImageError: cannot identify image file 'fig/axon_slice.jpg'
```

We can then check what type of variable is, using the `type()` function:

PYTHON < >

```
print(type(img))
```

OUTPUT < >

```
NameError: name 'img' is not defined
```

This tells us that the image is stored in a NumPy array; `ndarray` here refers to an N-dimensional array. We can check some other properties of this array, for example, what the image dimensions are, using the `.shape` attribute:

PYTHON < >

```
print(img.shape)
```

OUTPUT < >

```
NameError: name 'img' is not defined
```

This tells us that our image is composed of 2300 by 3040 data units, or *pixels* as we are dealing with an image. The total number of pixels in the image, is equivalent to its resolution. In digital imaging, a pixel is simply a numerical value that represents either intensity, in a greyscale image; or colour in a colour image. Therefore, as data, a two-dimensional (2-D) greyscale image comprises a 2-D array of these intensity values, or elements; hence the name 'pixel' is an amalgamation of 'picture' and 'element'. The array in our example has two dimensions, and so we can expect the image to be 2-D as well. Let us now use matplotlib.pyplot's `imshow` function to plot the image to see what it looks like. We can set the colour map to `gray` in order to overwrite the default colour map.

PYTHON < >

```
from matplotlib.pyplot import subplots, show

fig, ax = subplots(figsize=(25, 15))

ax.imshow(img, cmap='gray');
```

PYTHON < >

```
show()
```



`imshow` has allowed us to plot the NumPy array of our image data as a picture. The figure is divided up into a number of pixels, and each of these is assigned an intensity value, which is held in the NumPy array. Let's have a closer look by selecting a smaller region of our image and plotting that.

PYTHON < >

```
from matplotlib.pyplot import subplots, show

fig, ax = subplots(figsize=(25, 15))

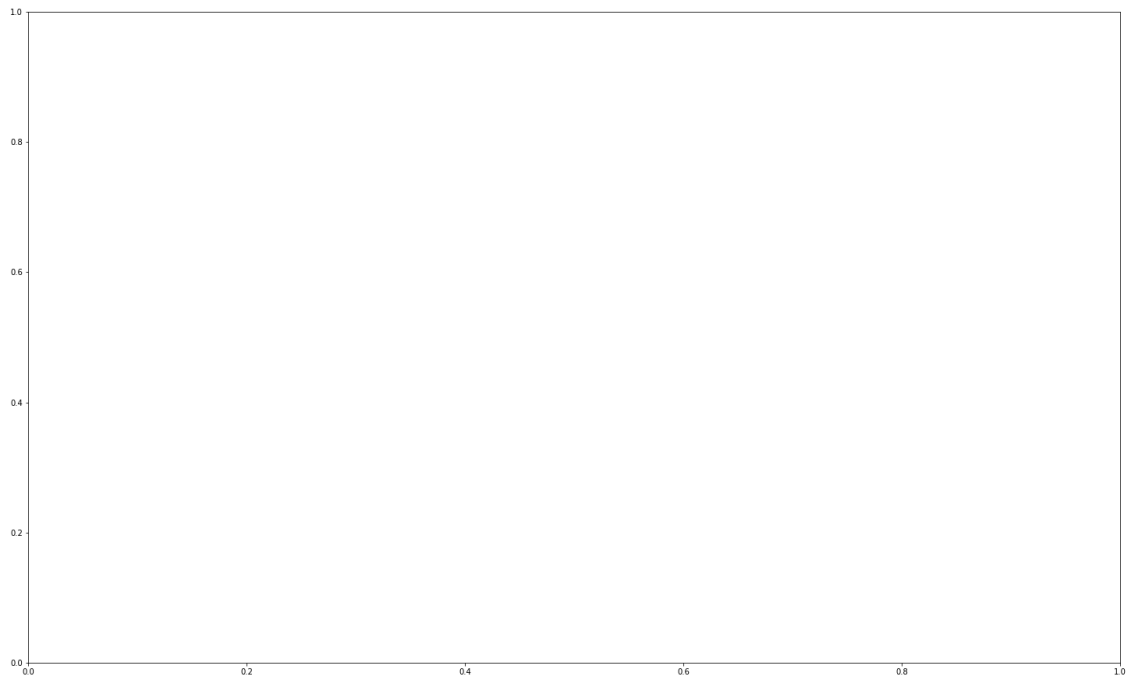
ax.imshow(img[:50, :70], cmap='gray');
```

OUTPUT < >

NameError: name 'img' is not defined

PYTHON < >

show()



By using `img[:50, :70]` we can select the first 50 values from the first dimension, and the first 70 values from the second dimension. Note that using a colon denotes 'all', where ':50' is inclusive of every number up to 50, for example. Thus, the image above shows a very small part of the upper left corner of our original image. As we are now zoomed in quite close to that corner, we can easily see the individual pixels here.

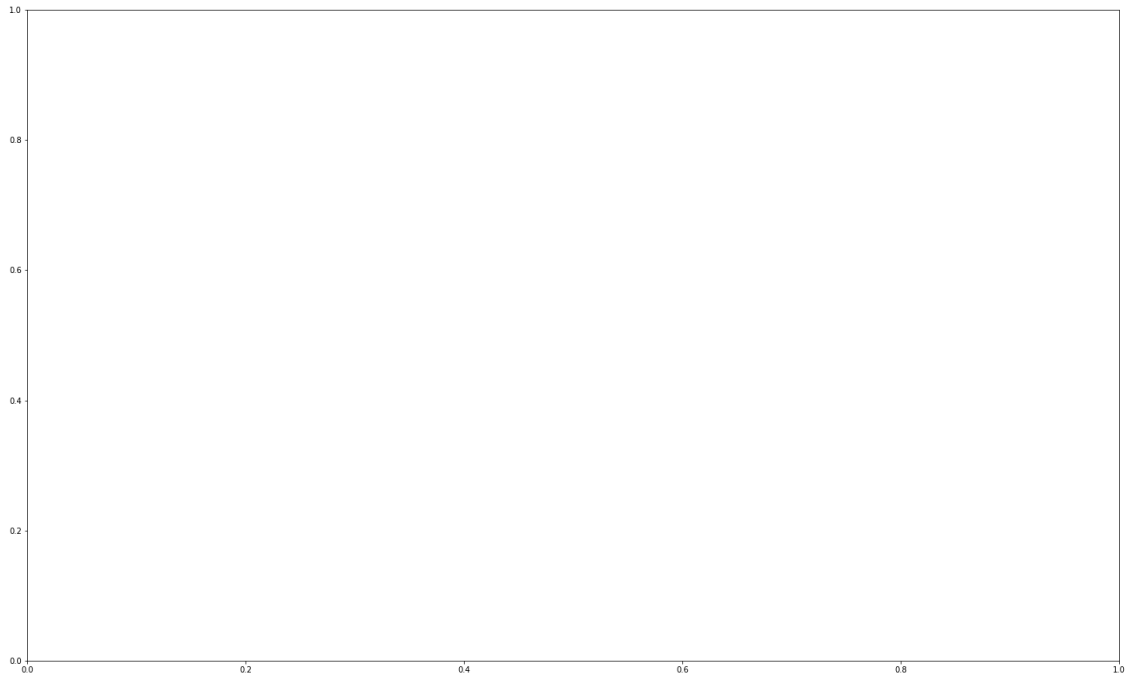
PYTHON < >

```
fig, ax = subplots(figsize=(25, 15))  
  
ax.imshow(img[:20, :15], cmap='gray');
```

OUTPUT < >

NameError: name 'img' is not defined

```
show()
```



We have now displayed an even smaller section from that same upper left corner of the image. Each square is a pixel and it has a single grey value. Thus, the pixel intensity values are assigned by the numbers stored in the NumPy array, `img`. Let us have a look at these values by producing a slice from the array.

```
print(img[:20, :15])
```

```
NameError: name 'img' is not defined
```

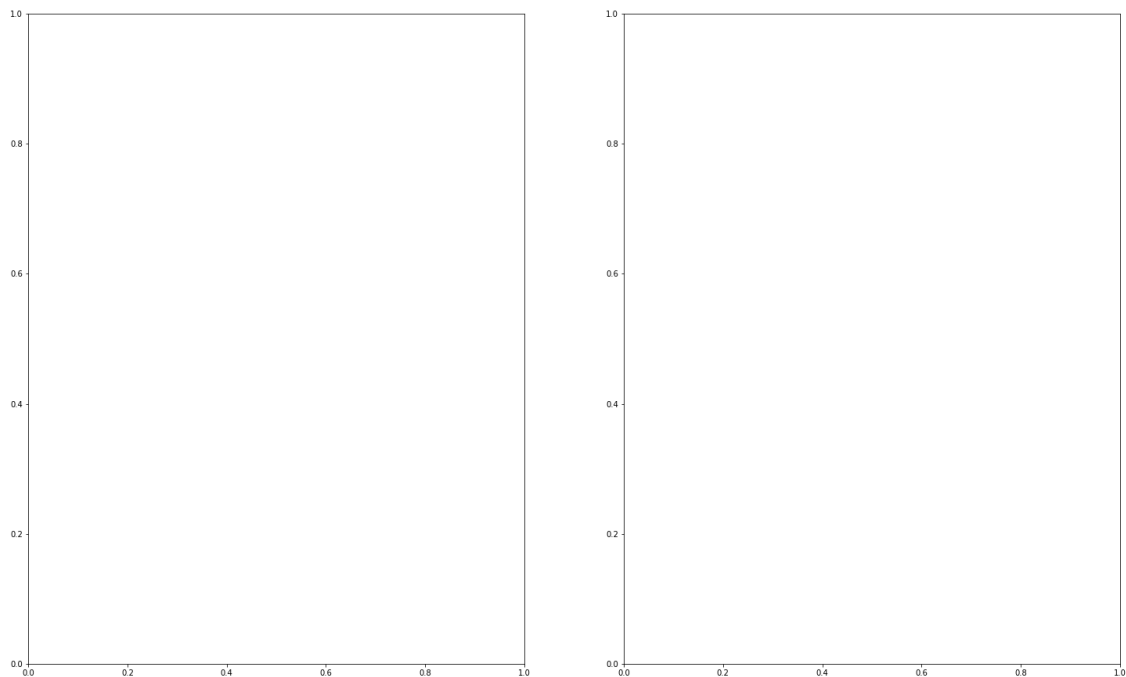
Each of these numbers corresponds to an intensity in the specified colourmap. These numbers range from 0 to 255, implying 256 shades of grey.

We chose `cmap = gray`, which assigns darker grey colours to smaller numbers, and lighter grey colours to higher numbers. However, we can also pick a non-greyscale colour map to plot the same image. We can even display a colourbar to keep track of the intensity values. Matplotlib has a diverse palette of colour maps that you can look through, linked [here](#). Let's take a look at two commonly-used Matplotlib colour maps called `viridis` and `magma`:

```
fig, ax = subplots(nrows=1, ncols=2, figsize=(25, 15))

p1 = ax[0].imshow(img[:20, :15], cmap='viridis')
p2 = ax[1].imshow(img[:20, :15], cmap='magma')
fig.colorbar(p1, ax=ax[0], shrink = 0.8)
fig.colorbar(p2, ax=ax[1], shrink = 0.8);

show()
```



Note, that even though we can plot our greyscale image with colourful colour schemes, it still does not qualify as a colour image. These are just false-colour, Matplotlib-overlaid interpretations of rudimentary intensity values. The reason for this is that a pixel in a standard colour image actually comprises **three sets** of intensities per pixel; not just the one, as in this greyscale example. In the case above, the number in the array represented a grey value and the colour was assigned to that grey value by **Matplotlib**. These represent 'false' colours.

## Creating an Image Mask

Now that we know that images essentially comprise arrays of numerical intensities held in a NumPy array, we can start processing images using these numbers.

As an initial approach, we can plot a histogram of the intensity values that comprise an image. We can make use of the `.flatten()` method to turn the original 2300 x 3040 array into a one-dimensional array with 6,992,000 values. This rearrangement allows the numbers within the image to be represented by a single column inside a matrix or DataFrame.

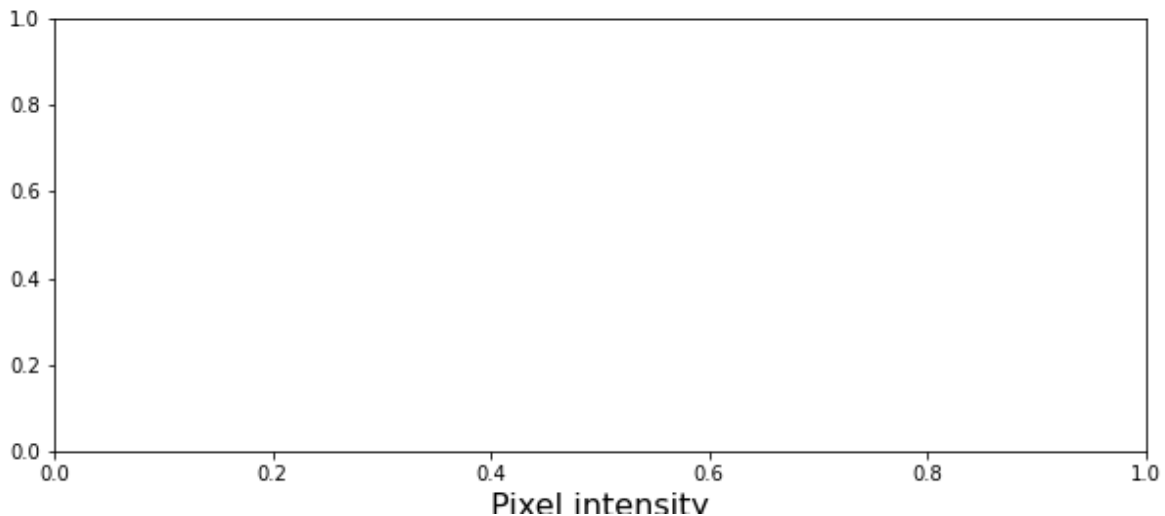
The histogram plot shows how several instances of each intensity make up this image:



```
fig, ax = subplots(figsize=(10, 4))

ax.hist(img.flatten(), bins = 50)
ax.set_xlabel("Pixel intensity", fontsize=16);

show()
```



The histogram is a distribution, with intensities mostly lying between values of 50 and 250.

The image displays a slice through an axon bundle. For the sake of example, let's say that we are now interested in the myelin sheath surrounding the axons (the dark rings). We can create a **mask** that isolates pixels whose intensity value is below a certain threshold (because darker pixels have lower intensity values). Everything below this threshold can be assigned to, for instance, a value of 1 (representing True), and everything above will be assigned a value of 0 (representing False). This is called a binary or Boolean mask.

Based on the histogram above, we might want to try adjusting that threshold somewhere between 100 and 200. Let's see what we get with a threshold set to 125. Firstly, we must implement a conditional statement in order to create the mask. It is then possible to apply this mask to the image. The result can be that we plot both the mask and the masked image.

```
threshold = 125

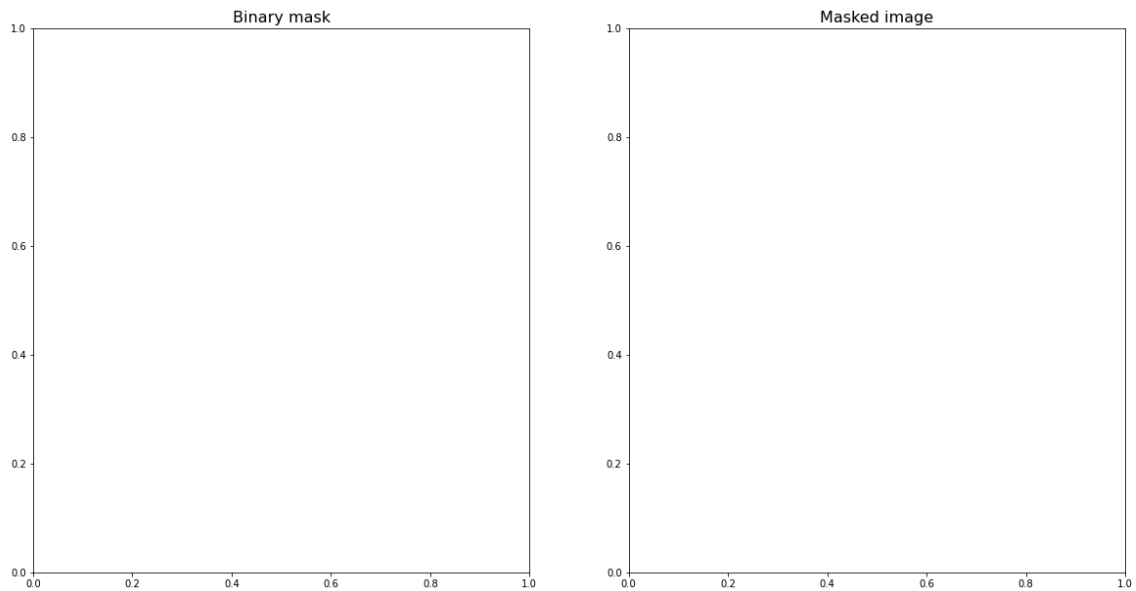
mask = img < threshold

img_masked = img*mask

fig, ax = subplots(nrows=1, ncols=2, figsize=(20, 10))

ax[0].imshow(mask, cmap='gray')
ax[0].set_title('Binary mask', fontsize=16)
ax[1].imshow(img_masked, cmap='gray')
ax[1].set_title('Masked image', fontsize=16)

show()
```



The left subplot shows the binary mask itself. White represents values where our condition is true, and black where our condition is false. The right image shows the original image after we have applied the binary mask, it shows the original pixel intensities in regions where the mask value is true.

Note that applying the mask means that the intensities where the condition is true are left unchanged. The intensities where the condition is false are multiplied by zero, and therefore set to zero.

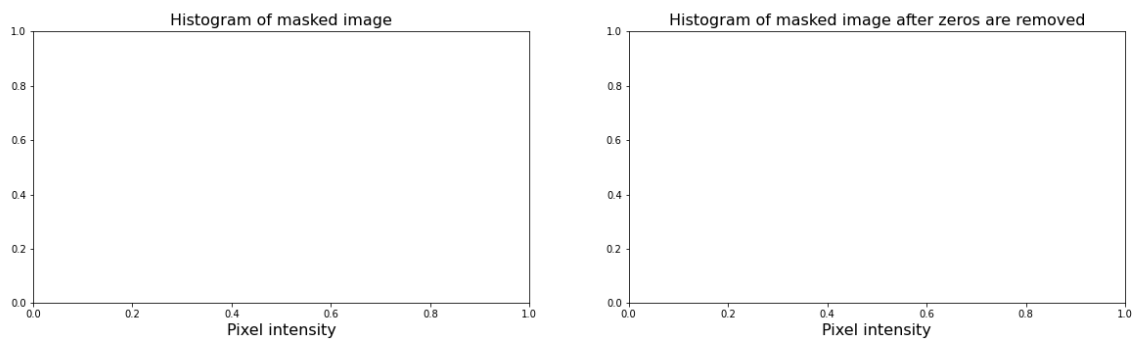
Let's have a look at the resulting image histograms.

```
fig, ax = subplots(nrows=1, ncols=2, figsize=(20, 5))

ax[0].hist(img_masked.flatten(), bins=50)
ax[0].set_title('Histogram of masked image', fontsize=16)
ax[0].set_xlabel("Pixel intensity", fontsize=16)

ax[1].hist(img_masked[img_masked != 0].flatten(), bins=25)
ax[1].set_title('Histogram of masked image after zeros are removed', fontsize=16)
ax[1].set_xlabel("Pixel intensity", fontsize=16)

show()
```



The left subplot displays the values for the masked image. Note that there is a large peak at zero, as a large part of the image is masked. The right subplot histogram, however, displays only the non-zero pixel intensities. From this, we can see that our mask has worked as expected, where only values up to 125 are found. This is because our threshold causes a sharp cut-off at a pixel intensity of 125.

## Colour Images

So far, our image has had a single intensity value for each pixel. In colour images, we have multiple channels of information per pixel; most commonly, colour images are RGB, comprising three corresponding to intensities of red, green and blue. There are many other formats of colour image, but these will be beyond the scope of this lesson. In a RGB colour image, any colour will be a composite of the intensity value for each of these colours. Our chosen example for a colour image is one of the rat cerebellar cortex. Let us firstly import it and check its shape.

```
img_col = imread('fig/rat_brain_low_res.jpg')
```

```
PIL.UnidentifiedImageError: cannot identify image file 'fig/rat_brain_low_res.jpg'
```

```
img_col.shape
```

[OUTPUT < >](#)

```
NameError: name 'img_col' is not defined
```

Our image array now contains three dimensions: the first two are the spatial dimensions corresponding to the pixel positions as x-y coordinates. The third dimension contains the three colour channels, corresponding to three layers of intensity values (red, blue and green) on top of each other, per pixel. First, let us plot the entire image using Matplotlib's imshow method.

First, let us plot the whole image.

[PYTHON < >](#)

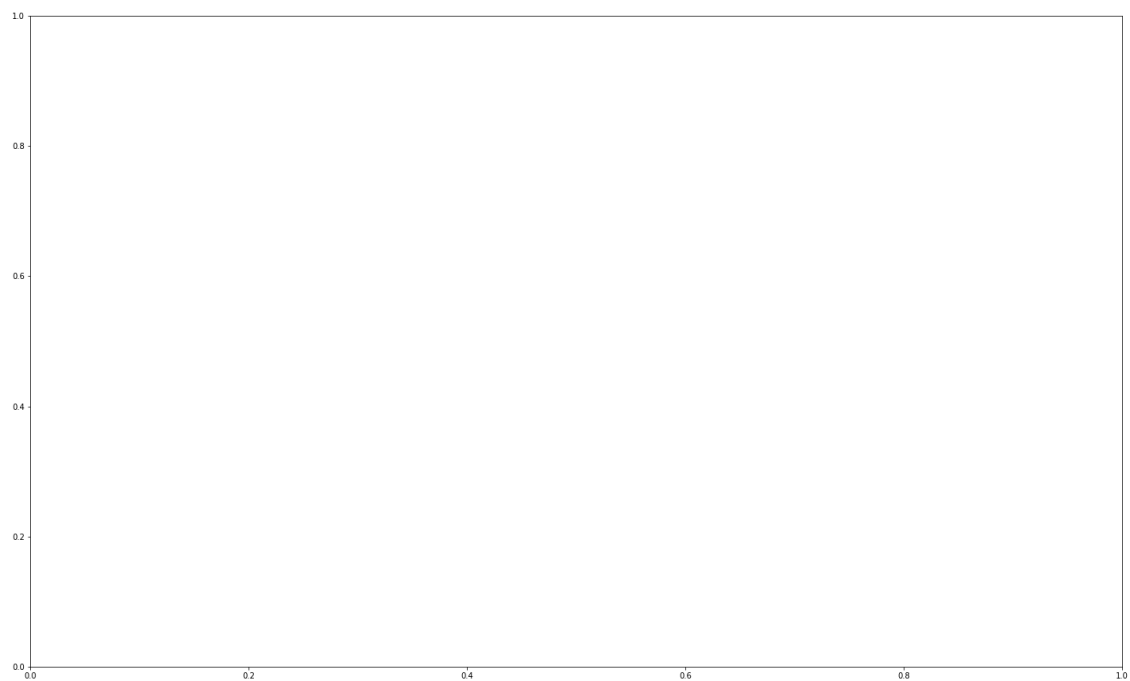
```
fig, ax = subplots(figsize=(25, 15))  
  
ax.imshow(img_col);
```

[OUTPUT < >](#)

```
NameError: name 'img_col' is not defined
```

[PYTHON < >](#)

```
show()
```



The sample is labeled for Hoechst stain (blue), the Inositol trisphosphate (IP3) receptor (green) and Glial fibrillary acidic protein (GFAP) (red).

We can then visualise the three colour channels individually by slicing the NumPy array comprising our image. The stack with index 0 corresponds to 'red', index 1 corresponds to 'green' and index 2 corresponds to 'blue':

PYTHON < >

```
red_channel = img_col[:, :, 0]
```

OUTPUT < >

```
NameError: name 'img_col' is not defined
```

PYTHON < >

```
green_channel = img_col[:, :, 1]
```

OUTPUT < >

```
NameError: name 'img_col' is not defined
```

PYTHON < >

```
blue_channel = img_col[:, :, 2]
```

OUTPUT < >

```
NameError: name 'img_col' is not defined
```

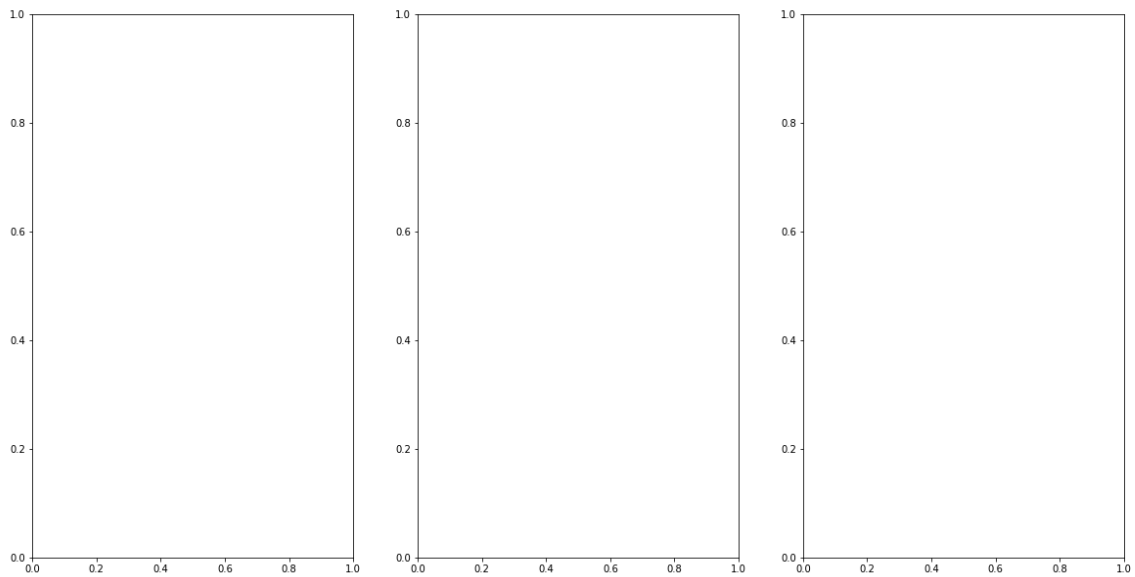
PYTHON < >

```
fig, ax = subplots(nrows=1, ncols=3, figsize=(20, 10))

imgplot_red = ax[0].imshow(red_channel, cmap="Reds")
imgplot_green = ax[1].imshow(green_channel, cmap="Greens")
imgplot_blue = ax[2].imshow(blue_channel, cmap="Blues")

fig.colorbar(imgplot_red, ax=ax[0], shrink=0.4)
fig.colorbar(imgplot_green, ax=ax[1], shrink=0.4)
fig.colorbar(imgplot_blue, ax=ax[2], shrink=0.4);

show()
```



This shows what colour combinations each of the pixels is made up of. Notice that the intensities go up to 255. This is because RGB (red, green and blue) colours are defined within an intensity range of 0-255. This gives a vast total 16,777,216 possible colour combinations.

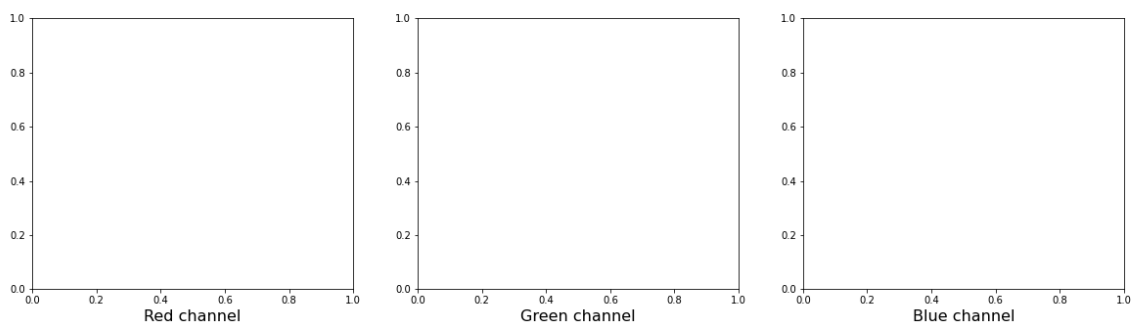
We can plot histograms of each of the colour channels.

PYTHON < >

```
fig, ax = subplots(nrows=1, ncols=3, figsize=(20, 5))

ax[0].hist(red_channel.flatten(), bins=50)
ax[0].set_xlabel("Pixel intensity", fontsize=16)
ax[0].set_xlabel("Red channel")
ax[1].hist(green_channel.flatten(), bins=50)
ax[1].set_xlabel("Pixel intensity", fontsize=16)
ax[1].set_xlabel("Green channel")
ax[2].hist(blue_channel.flatten(), bins=50)
ax[2].set_xlabel("Pixel intensity", fontsize=16)
ax[2].set_xlabel("Blue channel")

show()
```



## Dealing with Large Images

Often, we encounter situations where we have to deal with very large images that are composed of many pixels. Images such as these are often difficult to process, as they can require a lot of computer memory when they are processed. We will explore two different strategies for dealing with this problem - decreasing resolution, and using patches from the original image. To demonstrate this, we can make use of the full-resolution version of the rat brain image in the previous example.

PYTHON < >

```
img_hr = imread('fig/rat_brain.jpg')  
img_hr.shape
```

OUTPUT < >

```
PIL.UnidentifiedImageError: cannot identify image file 'fig/rat_brain.jpg'  
NameError: name 'img_hr' is not defined
```

When attempting to read the image in using the `imread()` function, we receive a warning from python indicating that the “Image size (324649360 pixels) exceeds limit of 178956970 pixels, could be decompression bomb DOS attack.” This is alluding to a potential malicious file, designed to crash or cause disruption by using up a lot of memory.

We can get around this by changing the maximum pixel limit, as follows.

To do this, we will need to use the Image module from the Python Image Library (PIL), as follows:

PYTHON < >

```
from PIL import Image  
  
Image.MAX_IMAGE_PIXELS = 1000000000
```

Let's try again. Please be patient, as this might take a moment.

PYTHON < >

```
img_hr = imread('fig/rat_brain.jpg')
```

OUTPUT < >

```
PIL.UnidentifiedImageError: cannot identify image file 'fig/rat_brain.jpg'
```

PYTHON < >

```
img_hr.shape
```

OUTPUT < >

NameError: name 'img\_hr' is not defined

Now we can plot the full high-resolution image:

PYTHON < >

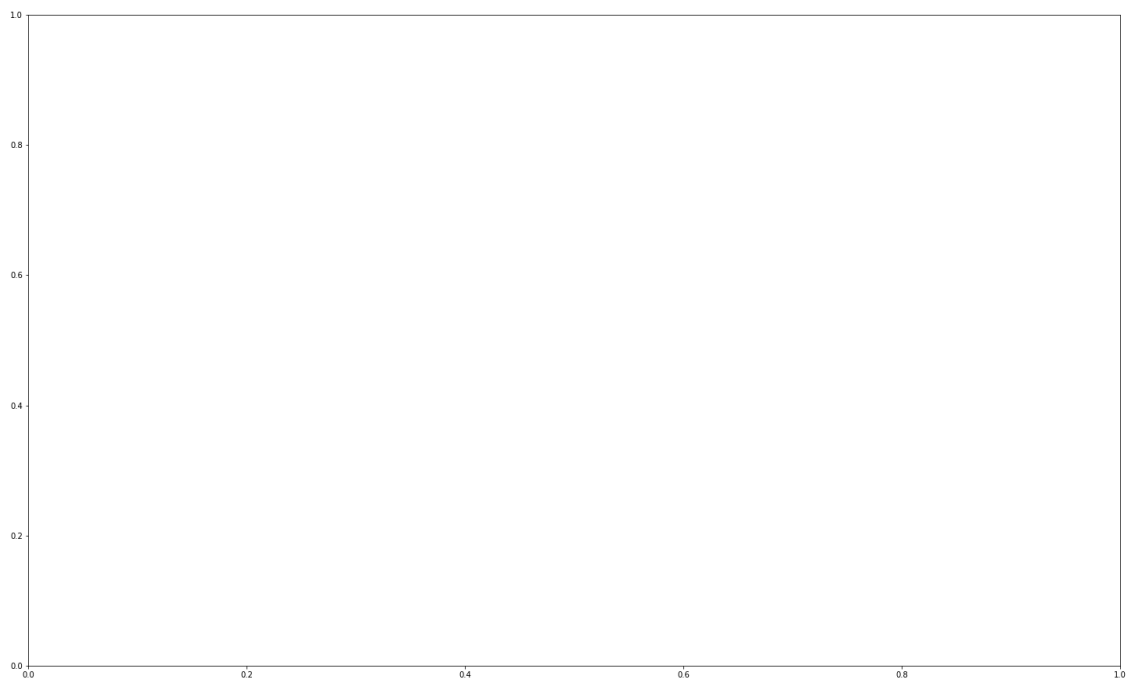
```
fig, ax = subplots(figsize=(25, 15))  
  
ax.imshow(img_hr, cmap='gray');
```

OUTPUT < >

NameError: name 'img\_hr' is not defined

PYTHON < >

```
show()
```



Although now we can plot this image, it does still consist of over 300 million pixels, which could run us into memory problems when attempting to process it. One approach is simply to reduce the resolution by importing the image using the Image module from the PIL library: which we imported in the code given, above. This library gives us a wealth of tools to process images, including methods to decrease an image's resolution. PIL is a very rich library with a multitude of



useful tools. As always, having a look at the official [documentation](#) and playing around with it yourself is highly encouraged.

We can make use of the **resize** method to downsample the image, providing the desired width and height as numerical arguments, as follows:

PYTHON < >

```
img_pil = Image.open('fig/rat_brain.jpg')
img_small = img_pil.resize((174, 187))

print(type(img_small))
```

OUTPUT < >

```
PIL.UnidentifiedImageError: cannot identify image file 'fig/rat_brain.jpg'
NameError: name 'img_pil' is not defined
NameError: name 'img_small' is not defined
```

Plotting should now be considerably quicker.

PYTHON < >

```
fig, ax = subplots(figsize=(25, 15))

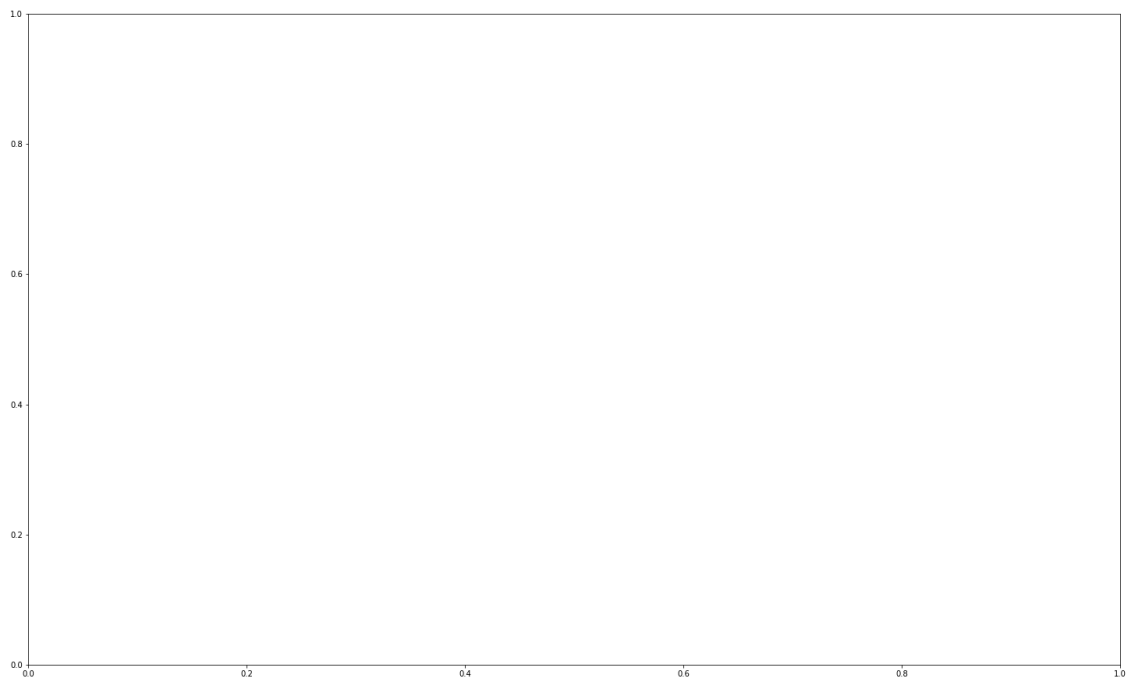
ax.imshow(img_small, cmap='gray');
```

OUTPUT < >

```
NameError: name 'img_small' is not defined
```

PYTHON < >

```
show()
```



Using the above code, we have successfully resized the image to a resolution of 174 x 187 pixels. It is, however, important to note that our image is no longer in the format of a NumPy array, but rather it now has the type `PIL . Image . Image`. We can, however, easily convert it back into a NumPy array using the `array` function, if we so wish.

PYTHON < >

```
from numpy import array  
  
img_numpy = array(img_small)
```

OUTPUT < >

```
NameError: name 'img_small' is not defined
```

PYTHON < >

```
print(type(img_numpy))
```

OUTPUT < >

```
NameError: name 'img_numpy' is not defined
```

Despite the above, it is often desired to keep images in their full resolution, as resizing effectively results in a loss of information. A commonly used, alternative approach to such downsampling is to **patch** the images. Patching an image

essentially divides the picture up into smaller chunks that are termed patches.

For this, we can implement functionality from the [Scikit-Learn](#) machine learning library.

PYTHON < >

```
from sklearn.feature_extraction.image import extract_patches_2d
```

The `extract_patches_2d` function is used to extract parts of the image. The shape of each patch, together with the maximal number of patches, can be specified, as follows:

PYTHON < >

```
patches = extract_patches_2d(img_hr, (174, 187), max_patches=100)
```

OUTPUT < >

```
NameError: name 'img_hr' is not defined
```

PYTHON < >

```
patches.shape
```

OUTPUT < >

```
NameError: name 'patches' is not defined
```

When using the `.shape` attribute, the output given here shows that the image has been successfully subdivided into 100 patches, with resolution 174 x 187 pixels, across three channels: red, blue and green (as this is an RGB, colour image). Note that patching itself can be a memory-intensive task. Extracting lots and lots of patches may take a long time. To look at the patches, we can make use of a for loop to iterate through the first element of the `.shape` array (the 100 patches), and visualise each one on its own subplot using the `.imshow` method, as follows:

PYTHON < >

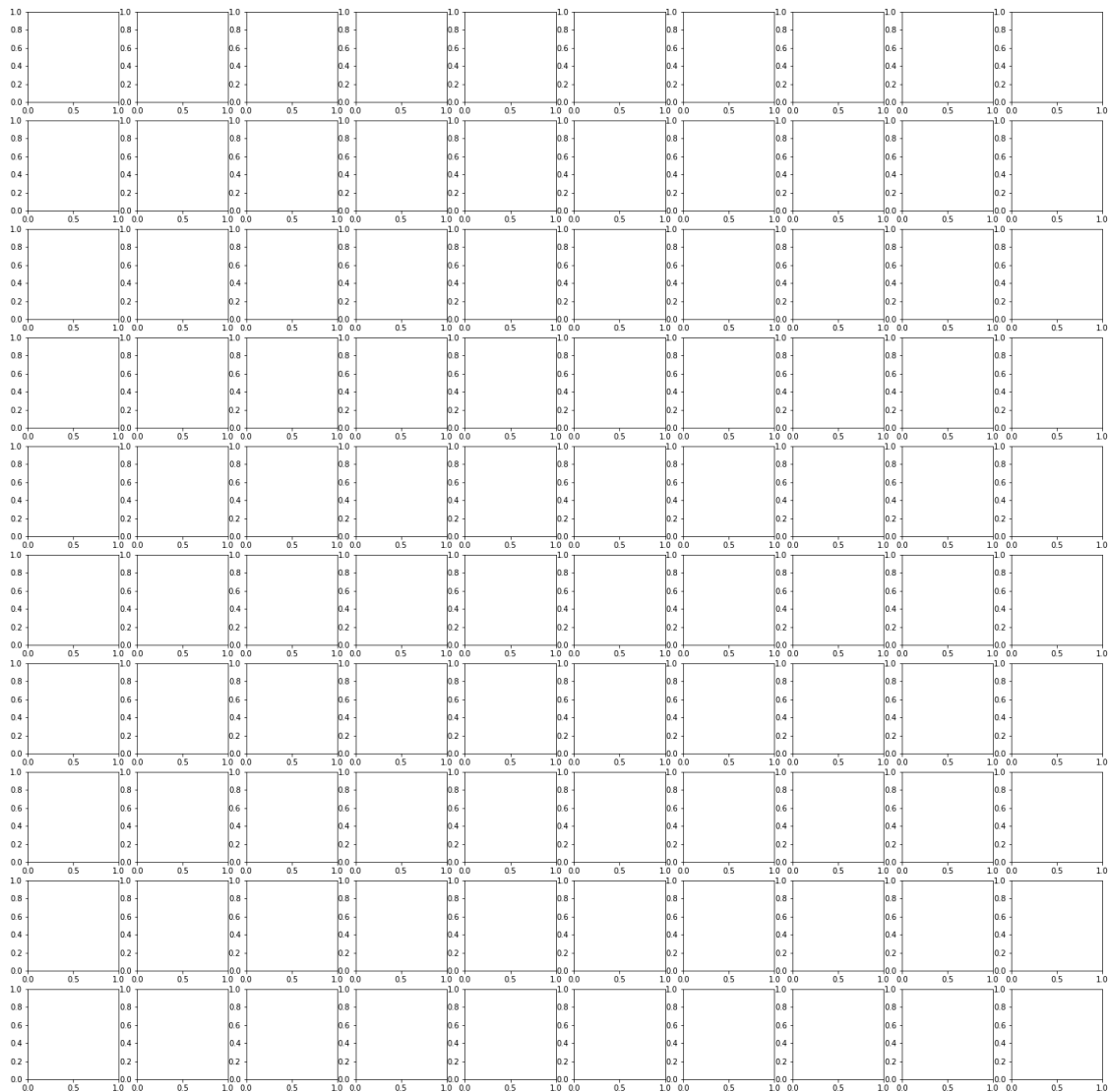
```
fig, ax = subplots(nrows=10, ncols=10, figsize=(25, 25))

ax = ax.flatten()

for index in range(patches.shape[0]):
    ax[index].imshow(patches[index, :, :, :])
```

NameError: name 'patches' is not defined

show()



Working with these smaller, individual patches will be much more manageable and computationally resourceful.

## 3D Images

So far, we have covered how to handle two-dimensional image data in a variety of ways. But scenarios will often arise whereby the analysis of three-dimensional image data is required. A good example of such 3-D images that is commonly encountered, is MRI scans. These don't natively exist as .csv format, but are often delivered in specialised image formats. One such format is nii: the **Neuroimaging Informatics Technology Initiative (NIfTI)** open file format. Handling these types of images requires specialised software. In particular, we will be using an open-source library called **nibabel**. Documentation for this package is available at <https://nipy.org/nibabel/>.

As it is not contained in your Python installation by default, it needs to be installed first.

Two methods for installing this, are given here. If you have previously installed the **Anaconda distribution** of Python, paste the following onto your command line or terminal, and hit return:

```
conda install -c conda-forge nibabel
```

Alternatively, you can also install it using Python's default package installer 'pip' by pasting the following onto your command line or terminal, and hitting return:

```
pip install nibabel
```

in your command line or terminal.

```
import nibabel as nib
```

PYTHON < >

The package is now available for use. To shorten how we call on the nibabel package in our code, we can use the above import statement, using the alias nib to refer to **nibabel**. Thus we can call on any function in nibabel, by using **nib** is followed by a period and the name of that specific function, as follows:

```
img_3d = nib.load('fig/brain.nii')  
  
img_data = img_3d.get_fdata()  
  
print(type(img_data))
```

PYTHON < >

```
<class 'numpy.memmap'>
```

OUTPUT < >

PYTHON < >

```
print(img_data.shape)
```

OUTPUT < >

```
(256, 256, 124)
```

We can see that the `.shape` attribute reveals that this image has three dimensions, and a total of 256 x 256 x 124 volume pixels (or voxels). To visualise our image, we can plot one slice at a time. The example below shows three different slices, in the transverse direction (from chin to the top of the head). To access an image across the transverse orientation, a single value is selected from the third dimension of the image, as follows. As always, the colons indicate that all data on the remaining two axes is selected and visualised:

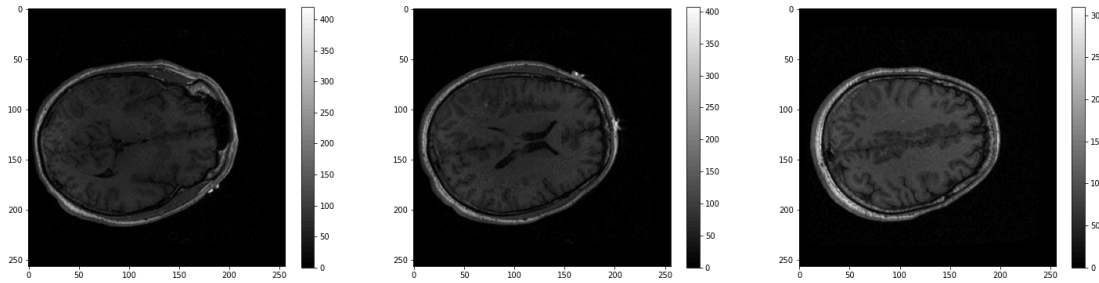
PYTHON < >

```
fig, ax = subplots(ncols=3, figsize=(25, 15))

p1 = ax[0].imshow(img_data[:, :, 60], cmap='gray')
p2 = ax[1].imshow(img_data[:, :, 75], cmap='gray')
p3 = ax[2].imshow(img_data[:, :, 90], cmap='gray')

fig.colorbar(p1, ax=ax[0], shrink=0.4)
fig.colorbar(p2, ax=ax[1], shrink=0.4)
fig.colorbar(p3, ax=ax[2], shrink=0.4);

show()
```



These look fairly dark. We can improve the contrast, by adjusting the intensity range. This requires intentionally overriding the default values, and specifying our own for the keyword arguments **vmin** and **vmax**.

The **vmin** and **vmax** parameters in matplotlib define the data range that the colormap (in our case the **gray** map) covers; thus they control the range of data values indicated by the colormap in use. By default, the colormap covers the complete value range of the supplied data. For instance, in many images this will typically be somewhere between 0 and 255. If we want to brighten up the darker shades of gray in our example MRI slices, for example, we can reduce the value of the **vmax** parameter.

Expanding upon the above code:

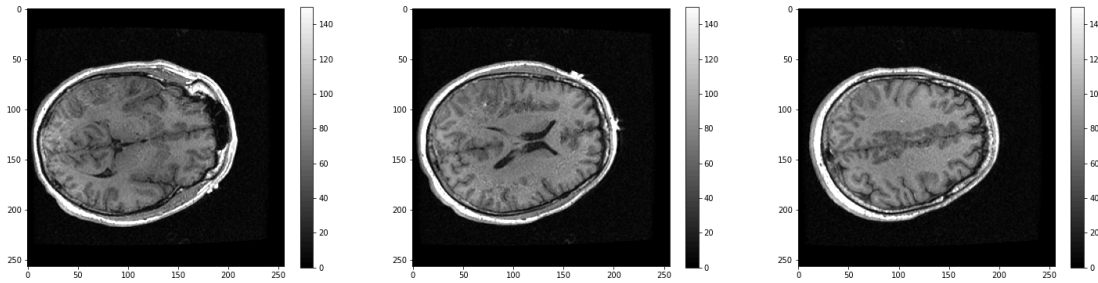
PYTHON < >

```
fig, ax = subplots(ncols=3, figsize=(25, 15))

p1 = ax[0].imshow(img_data[:, :, 60], cmap='gray', vmin=0, vmax=150)
p2 = ax[1].imshow(img_data[:, :, 75], cmap='gray', vmin=0, vmax=150)
p3 = ax[2].imshow(img_data[:, :, 90], cmap='gray', vmin=0, vmax=150)

fig.colorbar(p1, ax=ax[0], shrink=0.4)
fig.colorbar(p2, ax=ax[1], shrink=0.4)
fig.colorbar(p3, ax=ax[2], shrink=0.4);

show()
```



What about the other dimensions? We can also plot coronal and sagittal slices, for example. But take note that, in this case, the respective slices have different pixel resolutions. Let us expand our code even further, to display all three desired viewing planes in our MRI scan, as follows:

PYTHON < >

```
fig, ax = subplots(nrows=3, ncols=5, figsize=(26, 18))

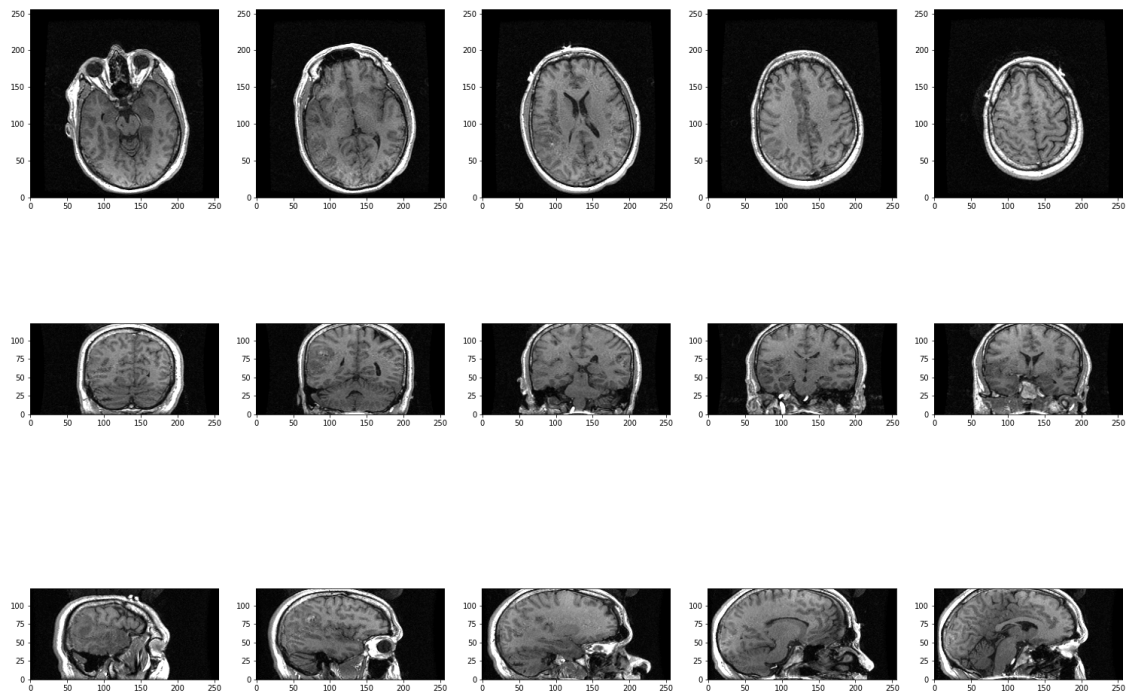
t1 = ax[0, 0].imshow(img_data[:, :, 45].T, cmap='gray', vmin=0, vmax=150, origin='lower')
t2 = ax[0, 1].imshow(img_data[:, :, 60].T, cmap='gray', vmin=0, vmax=150, origin='lower')
t3 = ax[0, 2].imshow(img_data[:, :, 75].T, cmap='gray', vmin=0, vmax=150, origin='lower')
t4 = ax[0, 3].imshow(img_data[:, :, 90].T, cmap='gray', vmin=0, vmax=150, origin='lower')
t5 = ax[0, 4].imshow(img_data[:, :, 105].T, cmap='gray', vmin=0, vmax=150, origin='lower')

c1 = ax[1, 0].imshow(img_data[:, 50, :].T, cmap='gray', vmin=0, vmax=150, origin='lower')
c2 = ax[1, 1].imshow(img_data[:, 75, :].T, cmap='gray', vmin=0, vmax=150, origin='lower')
c3 = ax[1, 2].imshow(img_data[:, 90, :].T, cmap='gray', vmin=0, vmax=150, origin='lower')
c4 = ax[1, 3].imshow(img_data[:, 105, :].T, cmap='gray', vmin=0, vmax=150, origin='lower')
c5 = ax[1, 4].imshow(img_data[:, 120, :].T, cmap='gray', vmin=0, vmax=150, origin='lower')

s1 = ax[2, 0].imshow(img_data[75, :, :].T, cmap='gray', vmin=0, vmax=150, origin='lower')
s2 = ax[2, 1].imshow(img_data[90, :, :].T, cmap='gray', vmin=0, vmax=150, origin='lower')
s3 = ax[2, 2].imshow(img_data[105, :, :].T, cmap='gray', vmin=0, vmax=150, origin='lower')
s4 = ax[2, 3].imshow(img_data[120, :, :].T, cmap='gray', vmin=0, vmax=150, origin='lower')
s5 = ax[2, 4].imshow(img_data[135, :, :].T, cmap='gray', vmin=0, vmax=150, origin='lower');

show()
```





The result is that we have clearly visualised slices of our 3-D image, representing all three viewing planes in this 3-dimensional brain scan.

## Exercises

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## END OF CHAPTER EXERCISES

### Assignment

Using the image from the beginning of this lesson (rat\_cerebellum.jpg) to perform the following tasks:

1. Import the image and display it.
2. Show histograms for each of the colour channels and plot the contributions of each of the RGB colours to the final image, separately.
3. Create three different binary masks using manually determined thresholds: one for mostly red pixels, one for mostly green pixels, and one for mostly blue pixels. Note that you can apply conditions that are either greater or smaller than a threshold of your choice.
4. Plot the three masks and the corresponding masked images.
5. Using your masks, approximate the relative amounts of synaptophysin, IP3R, and DNA in the image. To do this, you can assume that the number of red pixels represents synaptophysin, green pixels represents IP3R and blue pixels represent DNA. The results will vary depending on the setting of the thresholds. How do different threshold values change your results?
6. Change the resolution of your image to different values. How does the resolution affect your results?

### Solution

## KEY POINTS

- The `imread` function can be used to read in and interpret multiple image formats.
- Masking isolates pixels whose intensity value is below a certain threshold.
- Colour images typically comprise three channels (corresponding to red, green and blue intensities).
- Python Image Library (PIL) helps to set and raise default pixel limits for reading in and handling larger images.