

Digital Image Processing in Python

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<https://github.com/alonyan/DIP>

Workshop overview

- Day 1
 - Core concepts and motivation
 - What is a digital image
 - Pixel-level operations
- Day 2
 - Pixel-level operations (cont.)
 - Variance balancing (thresholding)
 - Image segmentation
- Day 3
 - Image segmentation (cont.)
 - Feature extraction
 - Cell tracking
 - Intro to modern computer vision

Workshop goals

The workshop is an introduction to digital image processing, designed to give you a taste of what's possible with a specific emphasis on microscopy data.

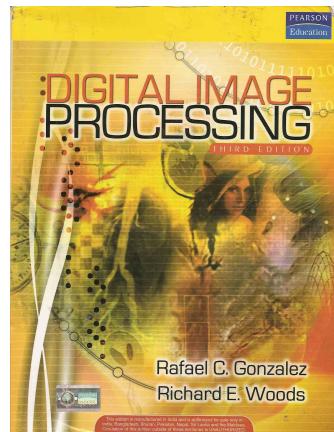
By the end of the workshop, you should:

- Acquire basic understanding and familiarity with computer vision.
- Appreciate the importance of rigorous and systematic image analysis for reproducible and quantitative science.

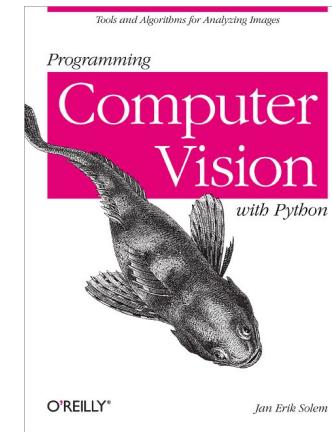
Other resources

MOOC: <https://www.mooc-list.com/course/image-analysis-methods-biologists-futurelearn>

<https://www.youtube.com/watch?v=1xo4vi6Ub4I>



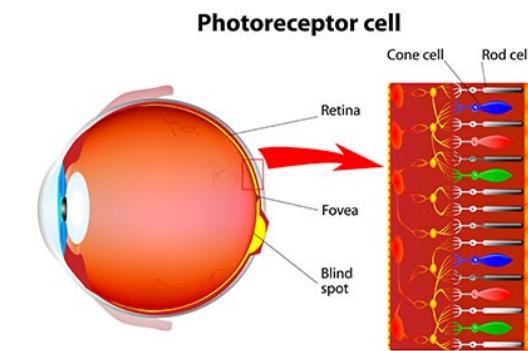
[http://web.ipac.caltech.edu/staff/fmasci/home/astro_refs/Digital Image Processing 2ndEd.pdf](http://web.ipac.caltech.edu/staff/fmasci/home/astro_refs/Digital_Image_Processing_2ndEd.pdf)



<http://programmingcomputervision.com/>

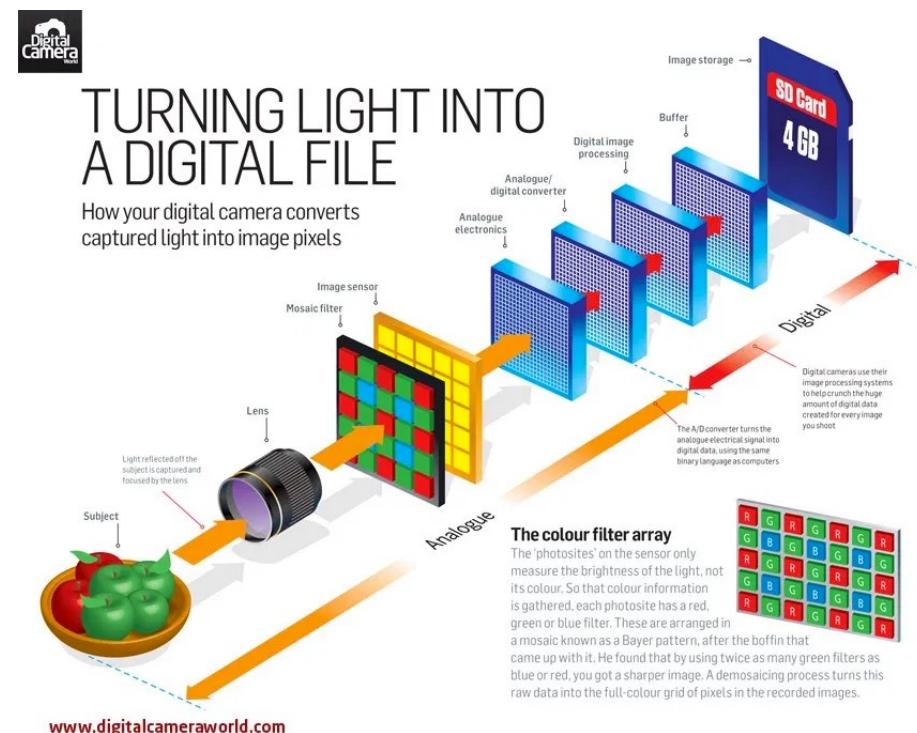
What is an Image?

- Photons are focused by lens (and cornea) and form an image on the retina.
- Activate a 2D array of photoreceptor cells that are sensitive to different colors.
- Information is passed to brain via optic nerves.



What is an Image?

- Photons are focused by a lens and form an image on an image sensor.
- Activate (charge) a 2D array of pixels on an image sensor (CCD/CMOS/PMT).
- (Sometimes there are color filters)
- Computers store information in bits , so the information must be digitized (ADC).
- Information is stored as a matrix of numbers.



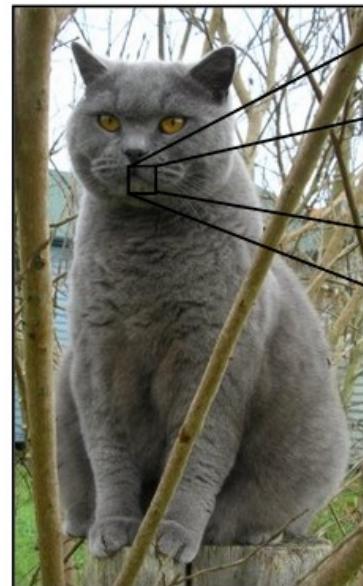
How is pixel data stored in the computer?

Data type	Range
uint8 – unsigned 8 bit integer	0 to 255
uint16 – unsigned 16 bit integer	0 to 65535
uint32 – unsigned 32 bit integer	0 to $2^{32} - 1$
float – floating point, single (32 bit) or double (64 bit) precision	-1 to 1 or 0 to 1
int8 - signed 8 bit integer	-128 to 127
int16 - signed 16 bit integer	-32768 to 32767
int32 - signed 32 bit integer	-2^{31} to $2^{31} - 1$

- The cost of higher precision is higher memory use.
- Higher precision is sometimes pointless, depending on the data and what you need to get from it.

What is an Image?

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```
08 02 22 97 38 15 00 40 00 75 04 05 07 78 52 12 50 77 01 00  
49 49 99 40 17 81 18 57 60 87 17 40 98 43 69 4 05 56 62 00  
01 49 31 73 55 79 14 29 93 71 40 67 51 18 30 03 49 13 36 65  
52 70 95 23 04 60 11 42 63 11 65 56 01 32 56 71 37 02 36 91  
22 31 16 71 51 67 05 89 41 92 36 54 22 40 40 28 66 33 13 80  
24 47 31 60 39 03 45 02 44 75 33 53 78 36 84 20 35 17 12 50  
32 98 81 28 64 23 67 10 26 38 40 67 59 54 70 66 18 38 64 70  
67 26 20 68 02 62 12 20 95 63 94 39 63 08 40 91 66 49 94 21  
24 55 38 05 66 73 99 26 97 17 78 78 96 83 14 80 34 89 63 72  
21 36 23 09 75 00 76 44 20 45 35 14 00 61 33 97 34 31 33 95  
78 17 53 28 22 75 31 67 15 94 03 80 04 62 16 14 09 53 56 92  
16 39 05 42 96 35 31 47 55 58 88 24 00 17 54 24 36 29 85 57  
86 56 00 48 35 71 89 07 05 44 44 37 44 50 21 58 51 54 17 58  
19 80 81 68 05 94 47 69 28 73 92 13 86 52 17 77 04 89 55 40  
04 52 08 83 97 35 99 16 07 97 57 32 16 26 26 79 33 27 98 66  
54 68 87 57 62 20 72 03 46 33 67 46 55 12 32 63 93 53 69  
04 42 16 73 36 65 39 11 24 94 72 18 05 46 29 32 40 62 76 36  
20 69 36 41 72 30 23 68 31 71 03 69 82 67 59 85 74 04 36 16  
20 73 35 29 72 31 90 01 74 31 49 71 45 05 44 23 57 05 54  
01 70 54 71 83 51 54 69 16 92 33 48 61 43 52 01 87 5 41
```

What the computer sees

Image Resolution

- Light propagates as a wave.
- Waves can have constructive or destructive interference.
- Lenses are designed to maximize constructive interference at the focal plane.

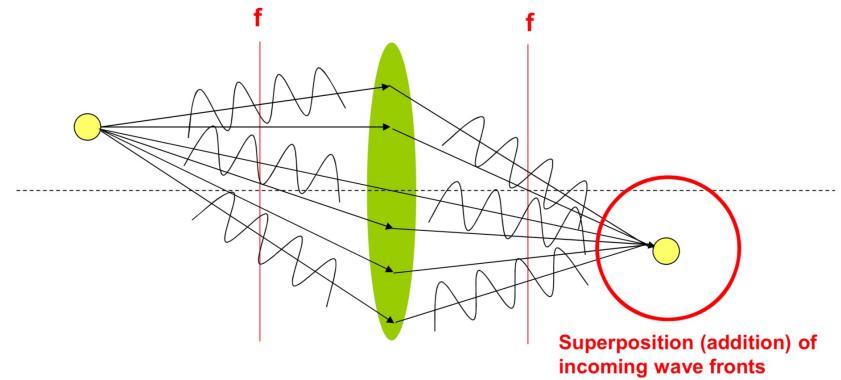


Image Resolution – Point Spread Function

- Image of point source - PSF

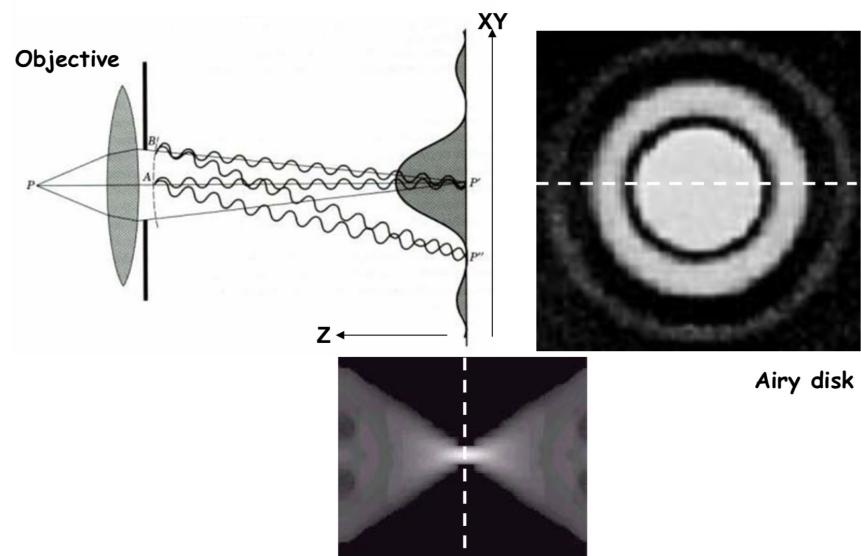


Image Resolution – Effect of Numerical Aperture

- $NA = n \cdot \sin \theta$
- Larger NA = Thinner PSF

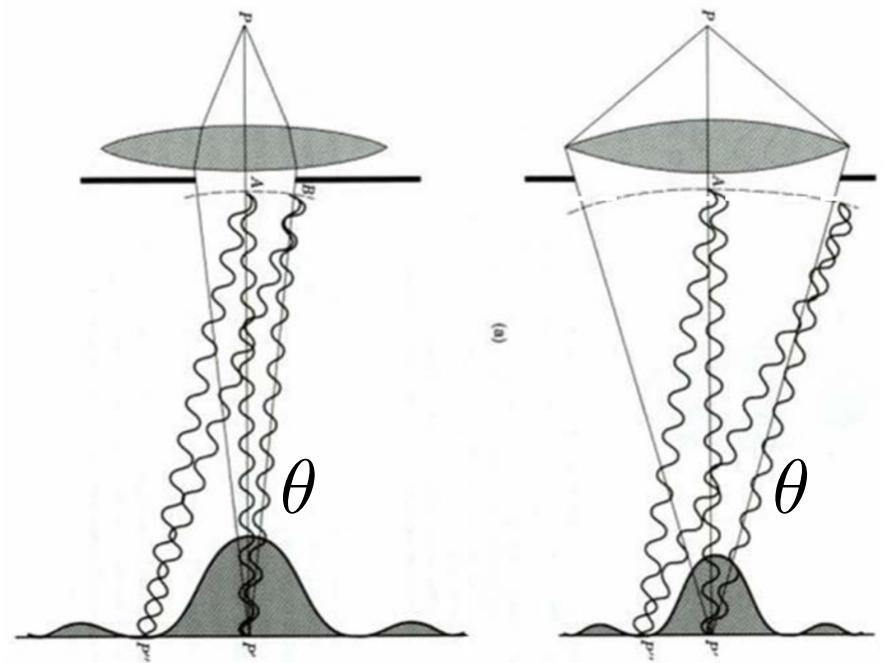
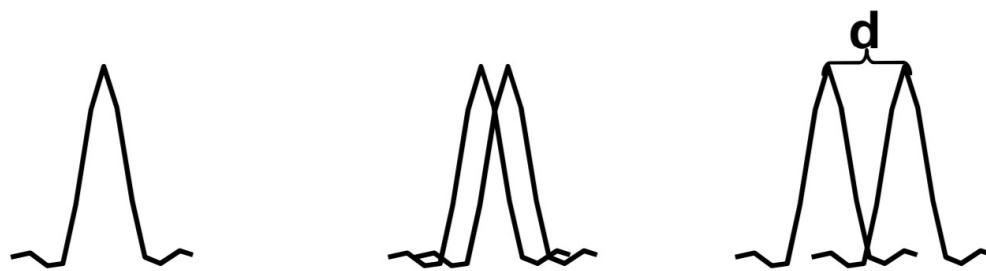
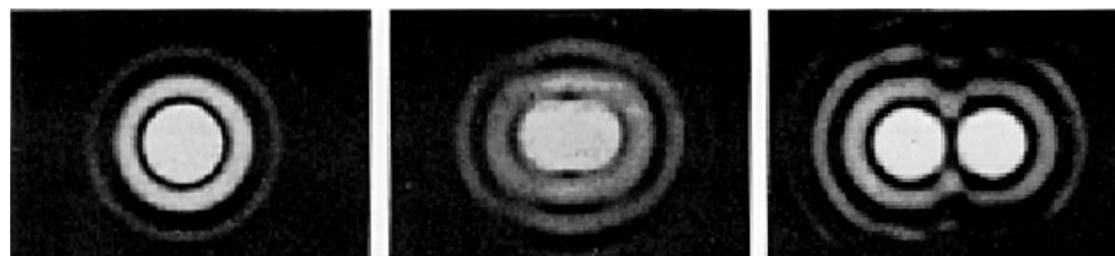


Image Resolution – How close two point can be and still be separable



$$\text{Lord Rayleigh's criterion: } \delta^R = 0.61 \frac{\lambda}{\text{NA}}$$

Physiologically motivated !!!

$$\delta_z^R = 2 \frac{\lambda n}{\text{NA}^2}$$

Aka Abbe
diffraction
limit

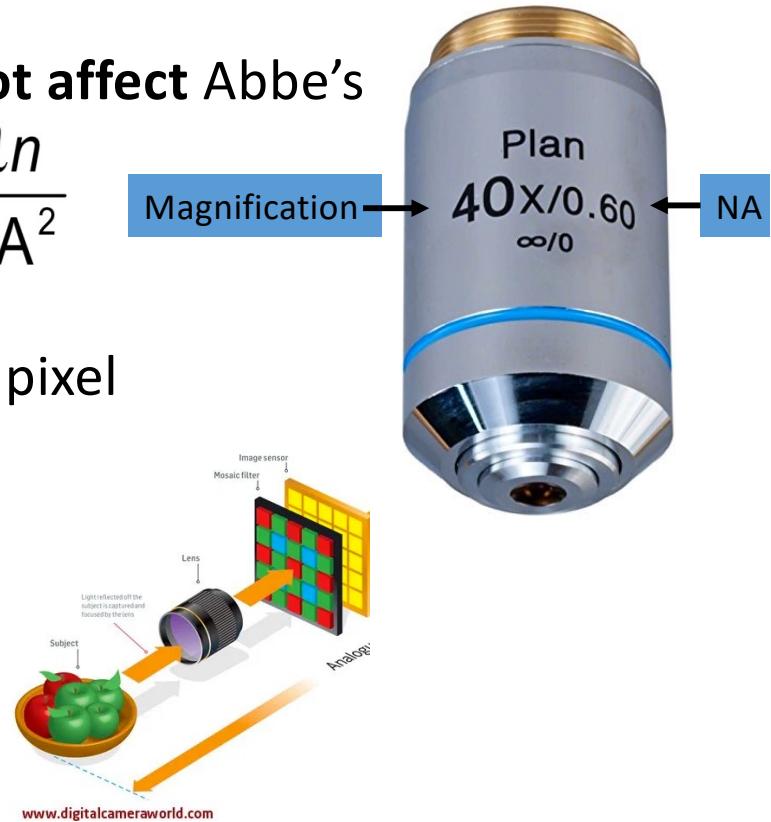
Image Resolution and magnification

- Notice that the objective magnification **does not affect** Abbe's resolution formulae:

$$\delta^R = 0.61 \frac{\lambda}{NA} \quad \delta_z^R = 2 \frac{\lambda n}{NA^2}$$

- Caveat: $\frac{\text{pixel size}}{\text{Mag}}$ is the area captured by each pixel in the camera.

If that is larger than Abbes limit than the resolution is set by that. In that case the system is not diffraction limited.



What is Image Processing?



Bunch of monkeys with
different attributes

- Our brain is really really good at this!
- Computer vision - teach **computers** to interpret and understand the visual world

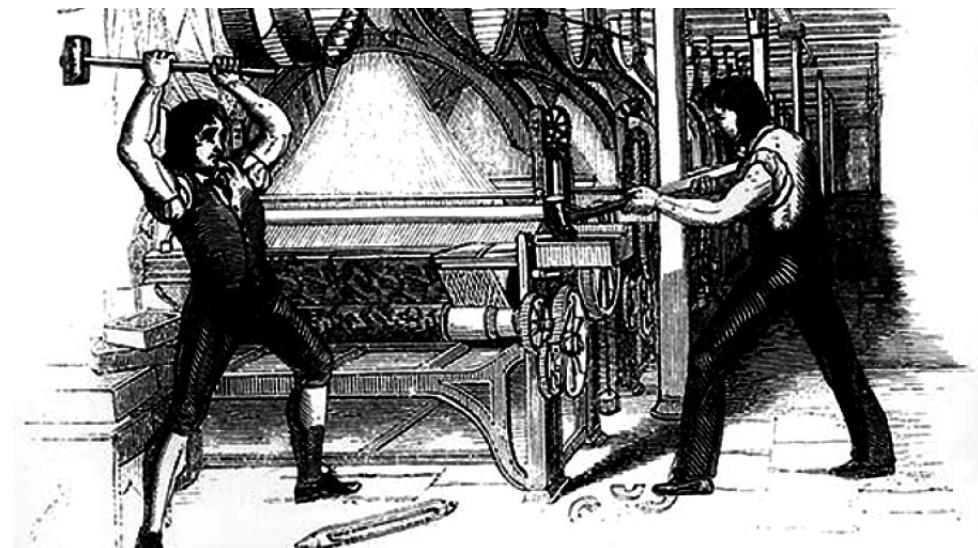
What is not Image Processing?



- Image processing is not magic
- Image processing doesn't create new data – that's called image manipulation/forgery
- It's always better to fix problems on the acquisition side than on the processing side
- **Good data is key!**

Why do we need image processing?

- If we're so good at this, why do we need computers?



(Optical) Microscopy circa y<2000 (b.f.p)

- Primarily antibody based
- Mostly fixed samples
- Low (throughput) and slow
- MB scale data,
typically ~10 images

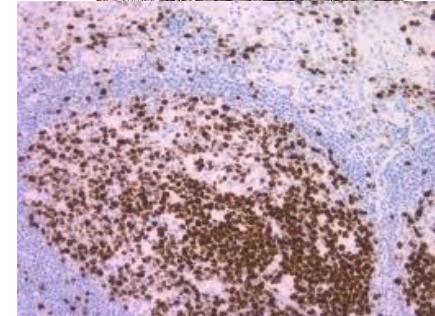
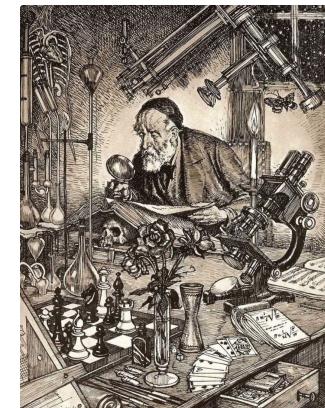


Image analysis was often performed manually,
usually on a subset of the data (“cherry picking”)
And heuristic methods that “make sense”

Microscopy circa y>2000 (a.f.p)

- FPs allow multichannel, live, long time imaging.
- New technologies:
 - Imaging beyond Abbe limit (STORM,PALM,STED,SIM...).
 - Imaging of whole organisms in 5D using LSFM (SPIM, Scanning beam, OPM,...) .
 - Multiplexed *in situ* RNA FISH (MERFISH, SeqFISH).
 - Multiplexed IF (CyCIF, MIBI)
 - Cryo-EM

The scale and type of data for the modern microscopist has exploded in the last 2 decades.

GB-TB scale data. In some cases $\sim 10^6$ Images/experiment. Manual image analysis becomes impractical.

Imaging has become a big data + high throughput problem.

Reproducibility

- Our goal is to extract meaningful, quantifiable information in a non-biased manner from imaging data.
- Manual, non-systematic, image analysis is prone to bias.



Computational Image processing

- Image processing is about *extracting* the relevant data from your measurements, but not about analyzing them.



Something
something
computers
...

Monkey ID	M/F	size	Coat Color	Attr 1	Attr 2	Attr 3	...
1	F	24	...				
2	F	40					
3	M	87					
4	M	21					
5	F	31					
6	F	24					
7	F	54					

What might an image processing pipeline look like?

Image acquisition



What might an image processing pipeline look like?

Image acquisition



Image Preprocessing

- Aligning
- Tiling
- Denoising

What might an image processing pipeline look like?

Image acquisition



Image Preprocessing

- Aligning
- Tiling
- Denoising

Image Processing

- Background / Foreground
- Segmentation
- Feature extraction
- Conversion to data

What might an image processing pipeline look like?

Image acquisition



Image Preprocessing

- Aligning
- Tiling
- Denoising

Image Processing

- Background / Foreground
- Segmentation
- Feature extraction
- Conversion to data

Data Analysis

- Anything that can be learned from the data

What kinds of images might we look at?

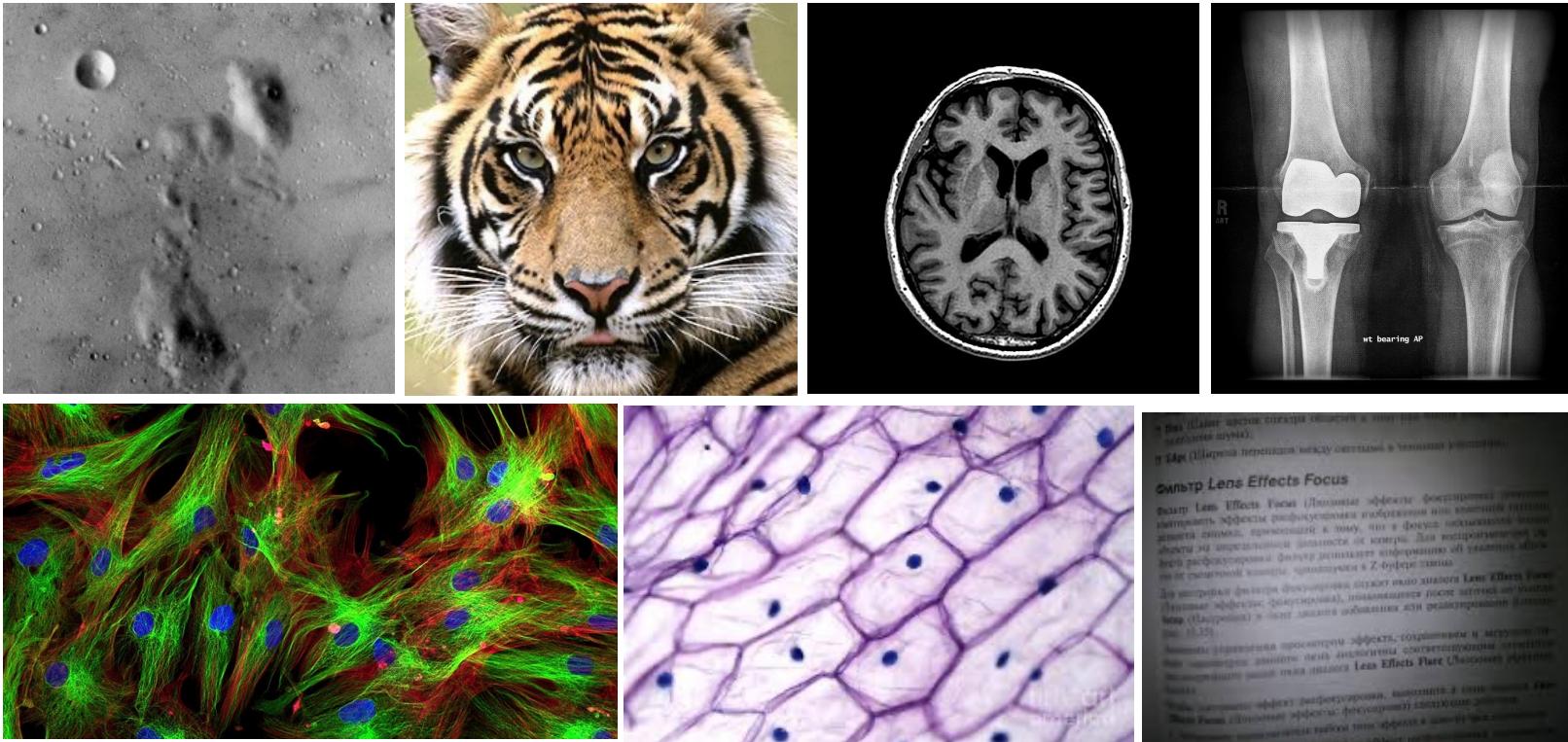


Image formats and compression

- It is important to have your data in the right format for the analysis you would like to perform.
- Some formats are considered **lossy** due to some compression when converting and saving in this format.
- Others are **lossless**, and uncompressed. This is more important when you would like to extract quantitative data from your image. They also tend to have larger file sizes.
- Many microscope manufacturers have their own proprietary image types and these will require specific tools to extract data from.

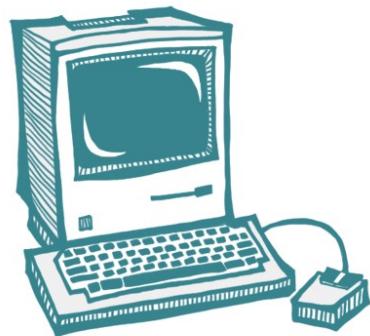
Some common data formats

- JPG or JPEG – Typically lossy and small bit depth (values range from 0 to 255). Can be grayscale or RGB, most common on standard digital cameras.
- PNG – Lossless image format but not high bit depth (0 to 255). Can also contain a transparency channel (alpha). One of the most common image formats.
- TIFF or TIF – Most common format in scientific imaging. Generally lossless and can have a bit depth up to 16 bit (0 to 65,535) in both grayscale and color. TIFF files can also be image stacks.
- CZI (zeiss) and LIF (Leica) – Proprietary image formats that can contain extra information about imaging and microscopy conditions.
- HDF5 / N5 - Hierarchical Data Format, designed to store very large amounts of structured data.

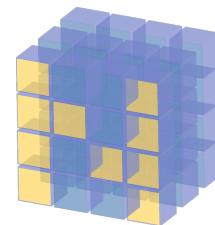
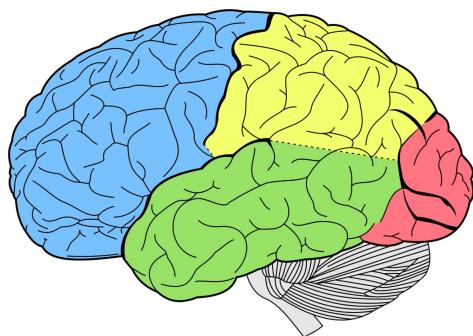
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Tools used in this workshop



scikit-image
image processing in python



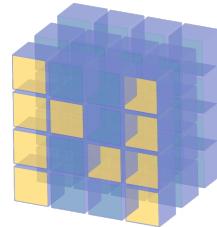
NumPy



Libraries we will be using

One of python's major advantages is it's extensive libraries that have been developed for a variety of different applications.

We'll be focusing this workshop on the libraries shown here.



NumPy



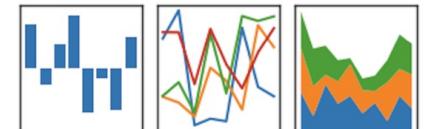
matplotlib

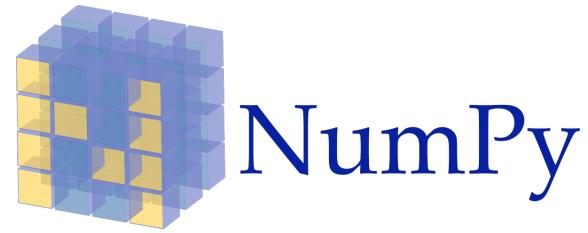


scikit-image
image processing in python

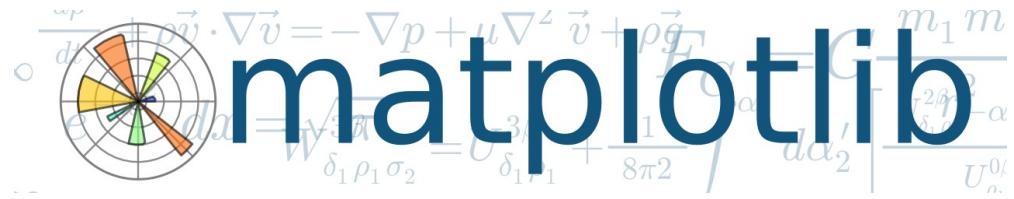
pandas

$$y_{it} = \beta' x_{it} + \mu_i + \epsilon_{it}$$





Advanced matrix calculator and
high-level math



Graphing, plotting, and viewing images

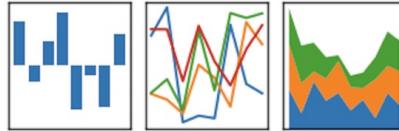


scikit-image
image processing in python

High-level image processing

pandas

$$y_{it} = \beta' x_{it} + \mu_i + \epsilon_{it}$$



Data management, manipulation and
analysis

What we'll be doing

The course has two tracks

1. Fully completed notebooks
2. Fill in the blanks notebooks

- You're strongly encouraged to work in pairs/groups, and to use google to look for answers. This is how this works in the real world!
- If you have basic experience programing, you should be able to fill in the blanks.
- If you don't, you can either tag along with someone who's more experienced, or just follow along with a completed notebook.

We'll be going over everything together, leaving time for you to try and figure stuff out on your own.

Working on your own data

- Test data is included as part of the workshop.
- Please only attempt to try your own data **after** you completed the task with the test data.
- My ability to debug your code will be limited

Setup

Let's get going!

We need to download/clone the relevant course material and access it using jupyter notebook

Cloning/Downloading the course repository

- Go to <https://github.com/alonyan/DIP>

The screenshot shows the GitHub repository page for 'alonyan / DIP'. The page includes the repository name, a search bar, navigation links for Pull requests, Issues, Marketplace, and Explore, and user statistics (ABD 9, 1). Below the header are buttons for Unwatch (1), Star (0), Fork (0), and Settings. The main content area displays repository details: UCLA QCBio Collaboratory workshop 21: Digital Image Analysis with Python, 2 commits, 1 branch, 0 releases, 1 contributor, and MIT license. It also shows a dropdown for Branch: master, a 'New pull request' button, and buttons for Create new file, Upload files, Find file, and Clone or download. A yellow callout box with the text 'Download the repo' and an arrow points to the 'Clone or download' button.

UCLA QCBio Collaboratory workshop 21: Digital Image Analysis with Python

Manage topics

2 commits 1 branch 0 releases 1 contributor MIT

Branch: master ▾ New pull request Create new file Upload files Find file Clone or download

Alon Oyler-Yaniv and Alon Oyler-Yaniv first commit

20190311 first commit 29 seconds ago

LICENSE Initial commit 8 minutes ago

README.md Initial commit 8 minutes ago

Download
the repo

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Cloning/Downloading the course repository

- Move the .ZIP file to wherever you want it (/Documents is a good spot) and unzip
- You can also navigate (cd) in the terminal/command line to wherever you want it and type:

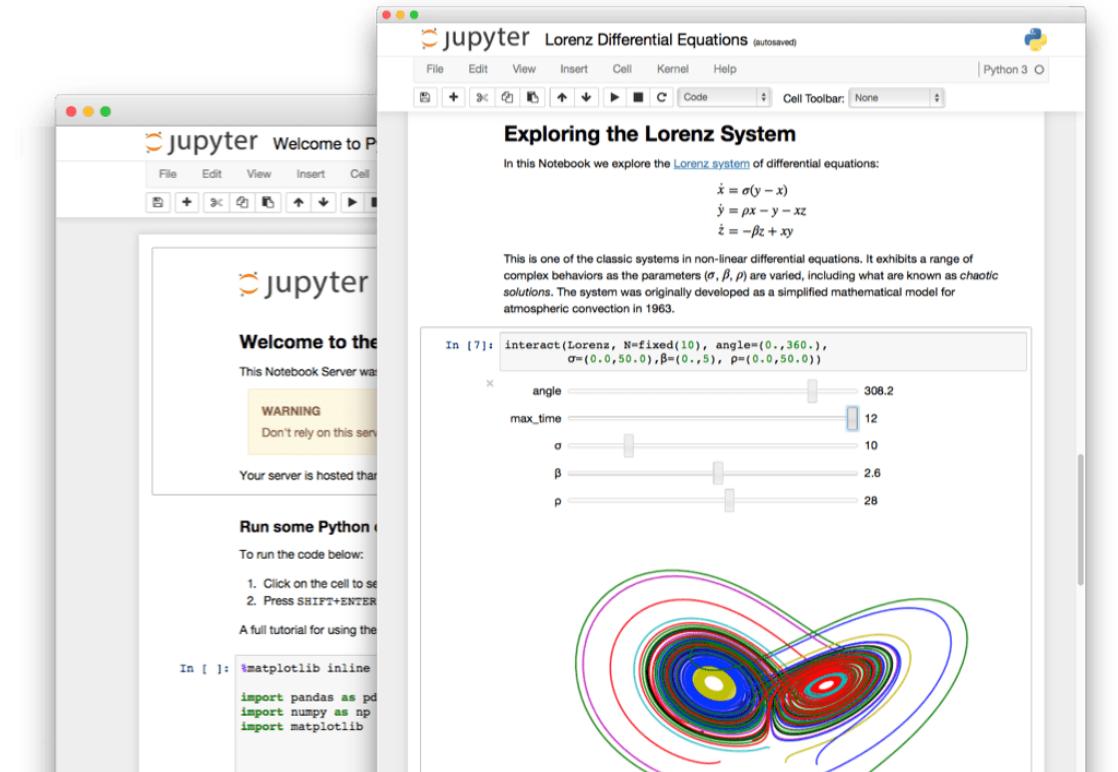
git clone <https://github.com/alonyan/DIP>

Getting started with Jupyter Notebooks

Jupyter notebooks

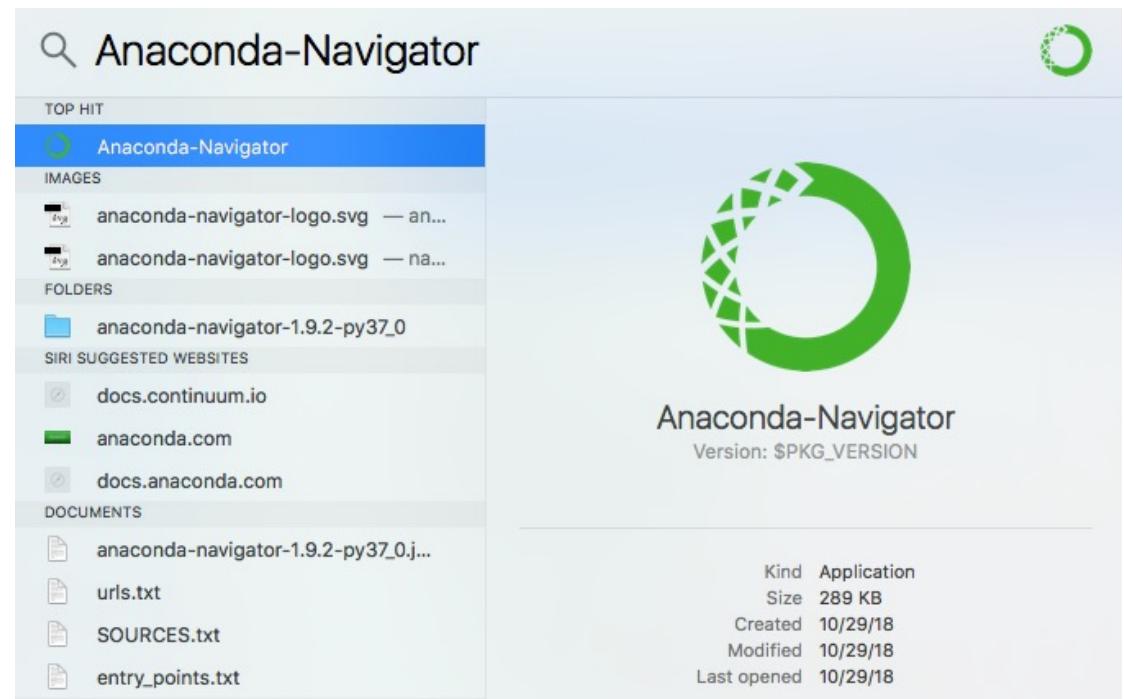
Jupyter notebooks are special documents that allow you to create and share documents **live code**.

They are a great way to keep your coding endeavors organized and share useful analysis with others.



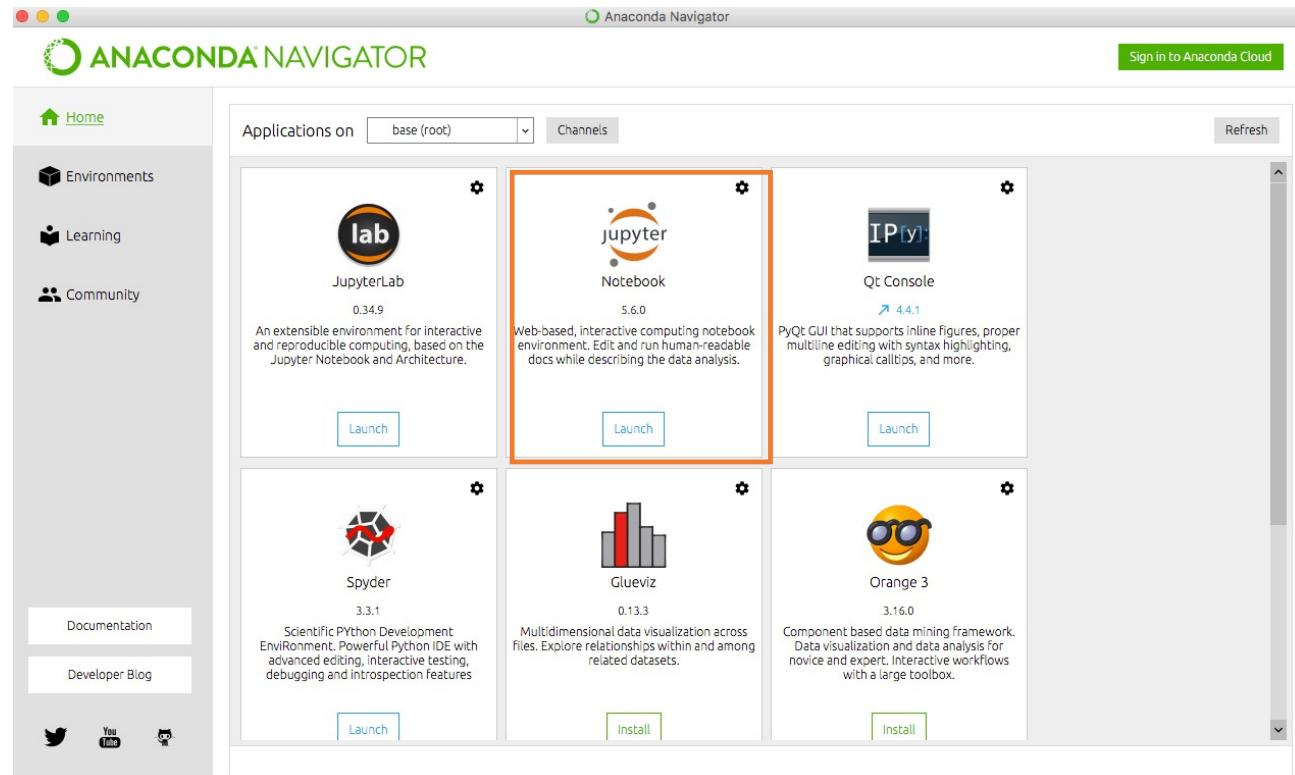
Getting started in Anaconda

- Launch the Anaconda application.
- MAC: Hit command+<space bar> and type “Anaconda”
- WINDOWS: Look for Anaconda on your start menu
- This should start you in the home directory.

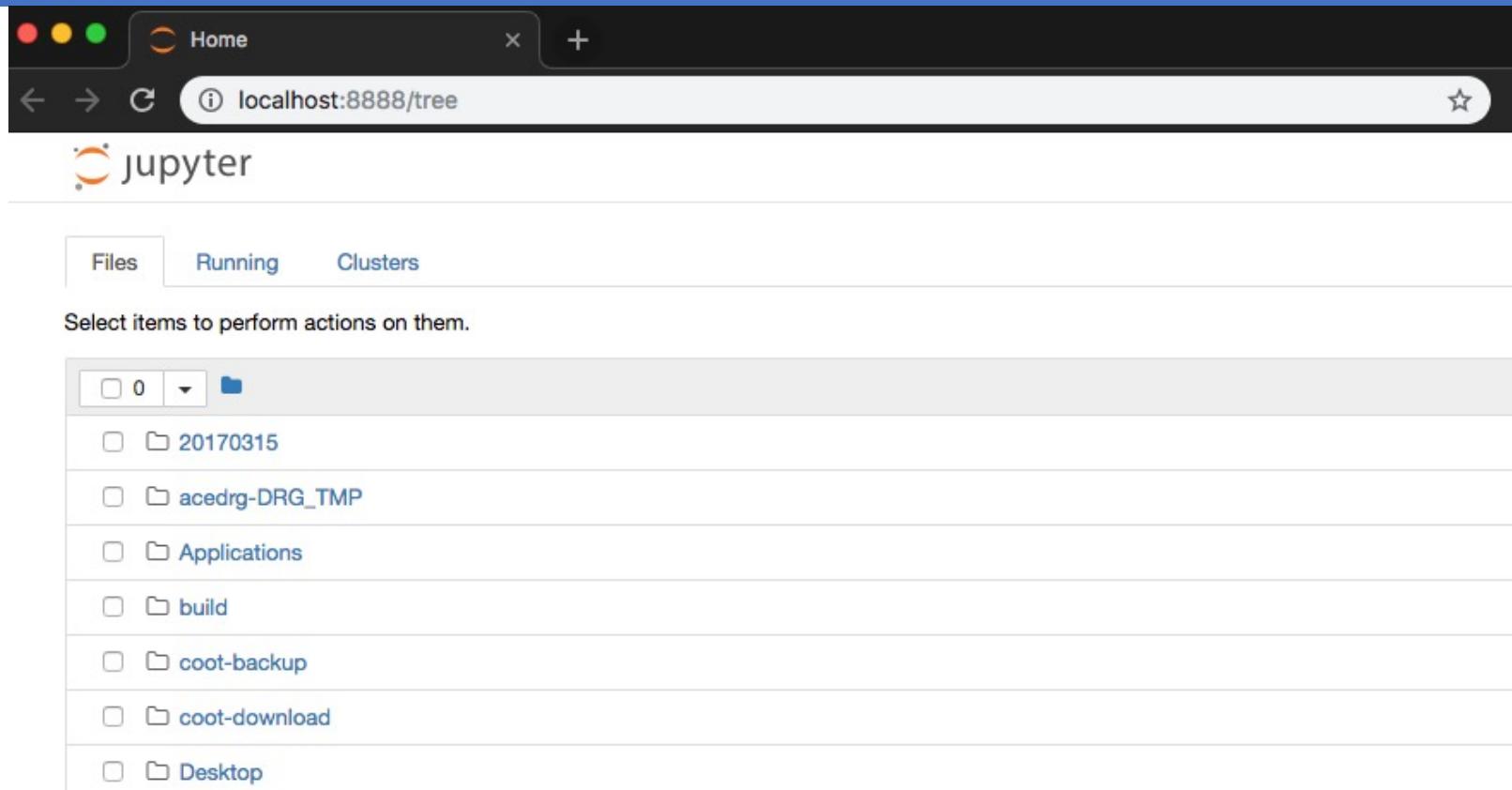


Getting started from Anaconda

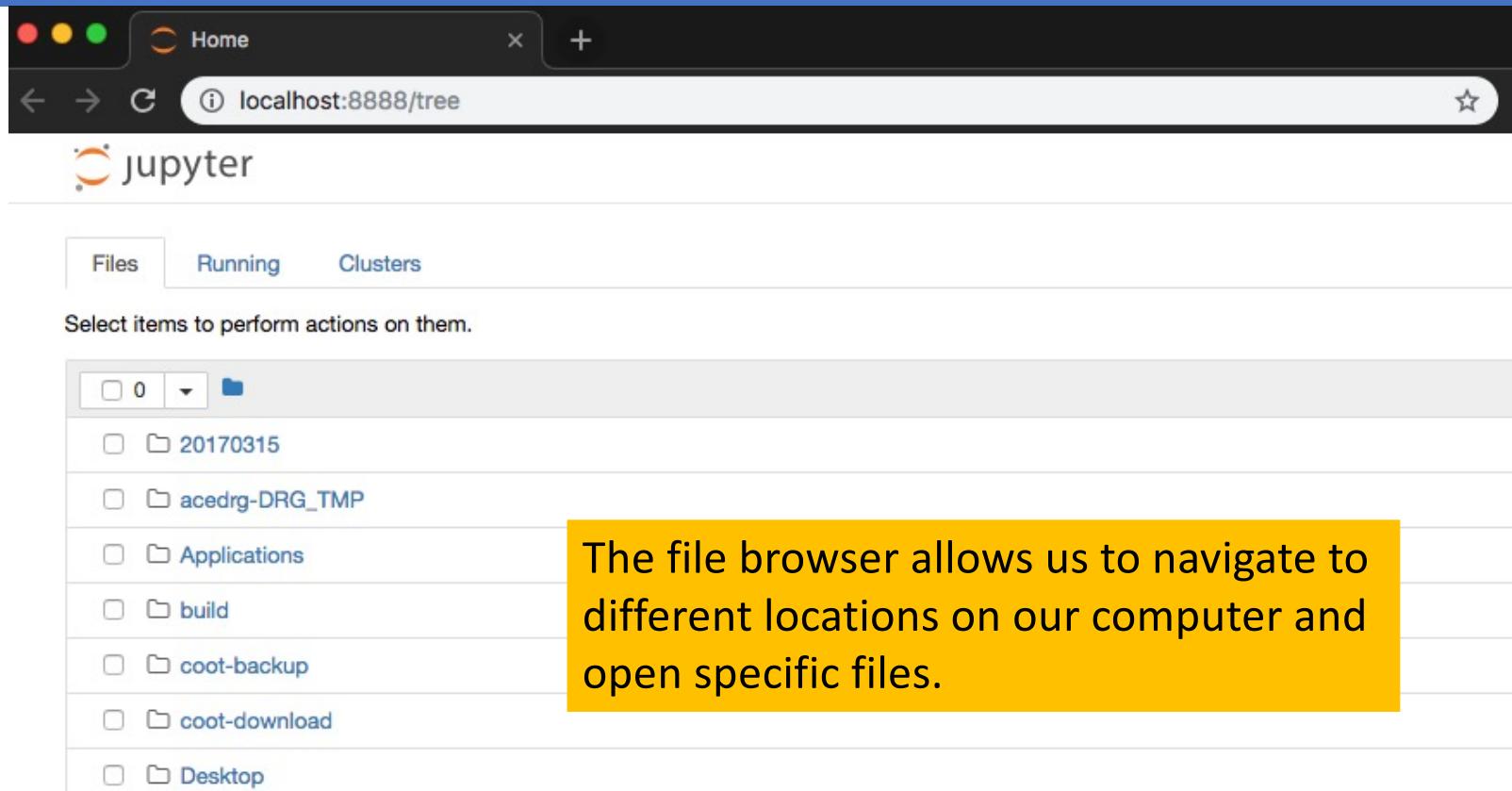
Click on the launch button under the **jupyter notebook** icon.
After a few moments the dashboard should open in your default web browser.



The jupyter dashboard



The jupyter dashboard

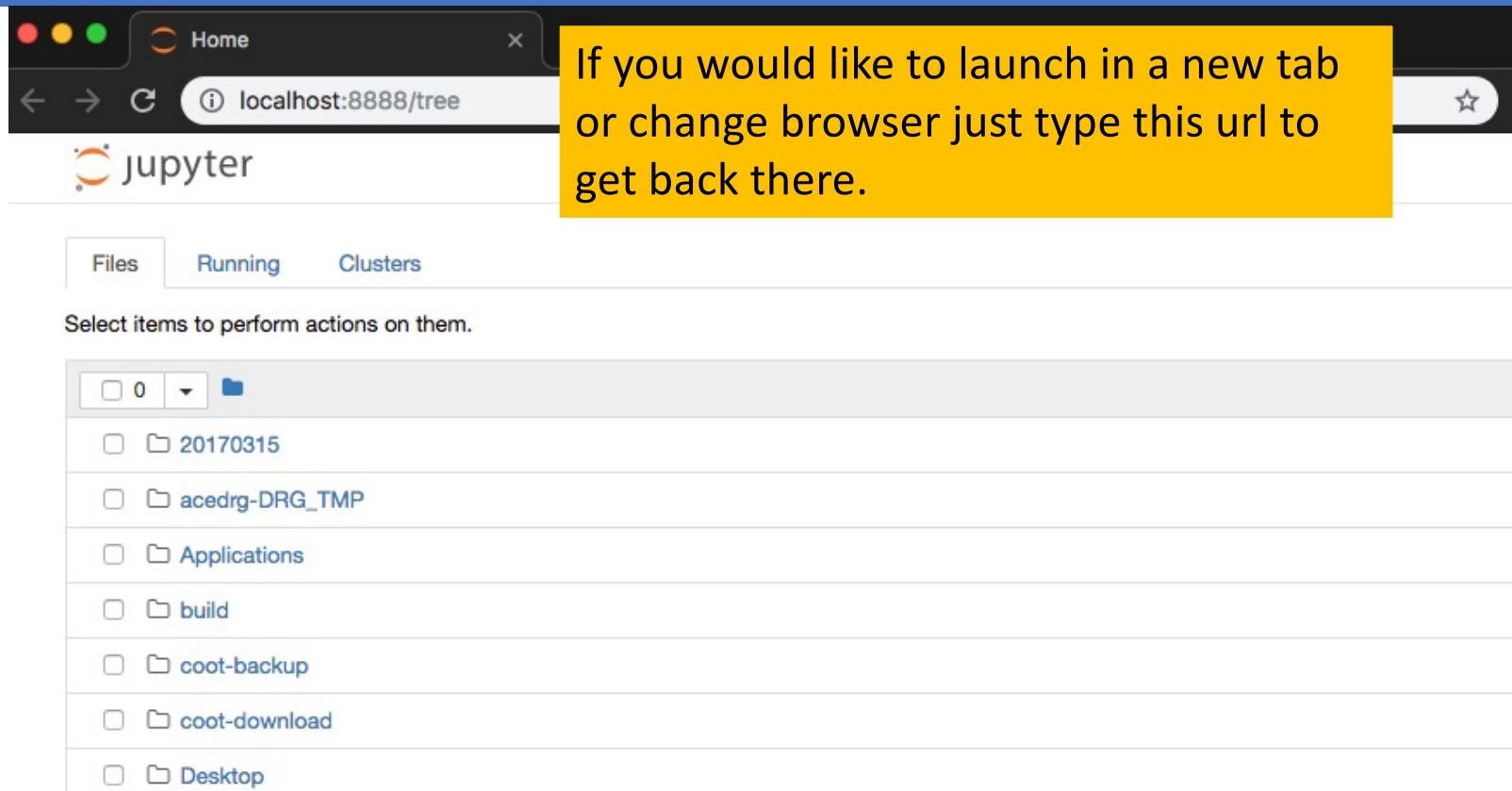


A screenshot of the Jupyter Notebook interface, specifically the file browser. The title bar shows "localhost:8888/tree". The main area has tabs for "Files", "Running", and "Clusters", with "Files" selected. A message "Select items to perform actions on them." is displayed above a file list. The file list shows the following structure:

- 0
- 20170315
- acedrg-DRG_TMP
- Applications
- build
- coot-backup
- coot-download
- Desktop

A yellow callout box highlights the file list area with the text: "The file browser allows us to navigate to different locations on our computer and open specific files."

The jupyter dashboard



The jupyter dashboard

Navigate to wherever you saved the repository

The screenshot shows the Jupyter Notebook dashboard. At the top, there's a dark header bar with a back arrow, a refresh icon, and the URL "localhost:8888/tree/Documents/Repos/DIP/20190311/JupyterNotebooks...". To the right of the URL are several icons: a star, ABP, a user count of 0, three dots, a 'F' icon, and a profile picture. Below the header is a yellow navigation bar with the text "Navigate to wherever you saved the repository". The main area has a title "jupyter" with an orange logo. Below it are tabs for "Files", "Running", and "Clusters", with "Files" being active. A message "Select items to perform actions on them." is displayed above a file list. The file list includes a search bar with "0" results, a folder icon, and the path "/ Documents / Repos / DIP / 20190311 / JupyterNotebooks". The columns in the file list are "Name", "Last Modified", and "File size". The file list contains four entries: a folder named ".." modified "seconds ago", a folder named "images" modified "10 minutes ago", a notebook named "DIP_AOY_Student.ipynb" modified "2 days ago" (size 53.3 kB), and another notebook named "DIP_AOY_Teacher.ipynb" modified "2 days ago" (size 7.8 MB). Action buttons "Upload", "New", and a refresh icon are located at the top right of the file list.

Name	Last Modified	File size
..	seconds ago	
images	10 minutes ago	
DIP_AOY_Student.ipynb	2 days ago	53.3 kB
DIP_AOY_Teacher.ipynb	2 days ago	7.8 MB

The jupyter dashboard

*.ipynb is the suffix of a notebook. Open the Student version (preferably) or the Teacher version of the nb.

The screenshot shows the Jupyter Notebook dashboard interface. At the top, there's a blue header bar with the title 'The jupyter dashboard'. Below it is a dark navigation bar with icons for back, forward, and search, followed by the URL 'localhost:8888/tree/Documents'. To the right of the URL are 'Quit' and 'Logout' buttons. The main area has tabs for 'Files', 'Running', and 'Clusters', with 'Files' currently selected. A yellow callout box contains the text: '*.ipynb is the suffix of a notebook. Open the Student version (preferably) or the Teacher version of the nb.' Below the tabs, there's a message 'Select items to perform actions on them.' and buttons for 'Upload', 'New ▾', and a refresh icon. The file list table has columns for selection, count (0), a dropdown menu, folder path ('/ Documents / Repos / DIP / 20190311 / JupyterNotebooks'), sorting options ('Name ↓', 'Last Modified', 'File size'), and file details ('seconds ago', '10 minutes ago', '2 days ago 53.3 kB', '2 days ago 7.8 MB').

	0	...	Name ↓	Last Modified	File size
<input type="checkbox"/>	0	...		seconds ago	
<input type="checkbox"/>	images			10 minutes ago	
<input type="checkbox"/>	DIP_AOY_Student.ipynb			2 days ago	53.3 kB
<input type="checkbox"/>	DIP_AOY_Teacher.ipynb			2 days ago	7.8 MB

localhost:8888/notebooks/Documents/Repos/DIP/20200505/JupyterNotebooks/DIP_AOY_Student.ipynb

jupyter DIP_AOY_Student Last Checkpoint: 01/14/2020 (autosaved)

Logout

File Edit View Insert Cell Kernel Widgets Help Trusted Python 3

disclaimer: To ensure that the notebook can be run from (more or less) any point, I try to load the relevant functions or modules whenever I use them in a cell. This is generally not good practice as it adds unnecessary overhead

0. Image representation as numerical arrays

You should see something like this

We start by importing numpy and c
array

In [1]:

```
%matplotlib inline
%load_ext autoreload
%autoreload
import numpy as np

#make a 9x9 checkerboard
checkBoard = #

print(checkBoard)
```

File "<ipython-input-1-149394df68h1>". line 7

Notebooks are divided into code blocks called “cells”.
To run all the code in a specific cell hit Shift+Enter

Python package structure – Modular programming

Package . Module . Function

Collection of modules Collection of functions Does a thing

Directory containing *.py files (and an __init__.py) Single *.py file Text within a *.py file

Import package **as pkg**

From package import module

From package.module import function

From module import *

pkg.module.function()

module.function()

function()

any_function_in_module()



quickmeme.com