Quantitative Big Imaging - Introduction

Anders Kaestner

CONTENTS

1	Toda	lys lecture	3
	1.1	We need some python modules	3
2	Abou	at the course	5
	2.1	Who are we?	5
	2.2	Who are you?	6
	2.3	So how will this ever work?	6
	2.4	Course Expectations	7
	2.5	Projects	7
	2.6	Course Overview	8
	2.7	Today's Reading Material	8
	2.8	Literature / Useful References	9
3	Moti	vation - You have data!	11
	3.1	Imaging experiments produce a lot of data	11
	3.2		12
	3.3		12
	3.4		13
	3.5		13
	3.6		14
4	Imag	ges	23
	4.1		23
	4.2	Let's create a small image	24
	4.3		
	T.J	2D Intensity Images	25
	4.4		25 35
		3D Images	
	4.4	3D Images	35
	4.4 4.5	3D Images	35 37
	4.4 4.5 4.6	3D Images	35 37 39
	4.4 4.5 4.6 4.7	3D Images Multiple Values per pixel Hyperspectral Imaging Image Formation Acquiring Images	35 37 39 41
5	4.4 4.5 4.6 4.7 4.8 4.9	3D Images Multiple Values per pixel Hyperspectral Imaging Image Formation Acquiring Images Traditional Imaging: Model	35 37 39 41 42
5	4.4 4.5 4.6 4.7 4.8 4.9	3D Images Multiple Values per pixel Hyperspectral Imaging Image Formation Acquiring Images Traditional Imaging: Model	35 37 39 41 42 44
5	4.4 4.5 4.6 4.7 4.8 4.9	3D Images Multiple Values per pixel Hyperspectral Imaging Image Formation Acquiring Images Traditional Imaging: Model Prent views on image Analysis Image Analysis: Experimentalist	35 37 39 41 42 44
5	4.4 4.5 4.6 4.7 4.8 4.9 Diffe 5.1	3D Images Multiple Values per pixel Hyperspectral Imaging Image Formation Acquiring Images Traditional Imaging: Model Prent views on image Analysis Image Analysis: Experimentalist Image Analysis: Computer Vision Approaches	35 37 39 41 42 44 47 48
5	4.4 4.5 4.6 4.7 4.8 4.9 Diffe 5.1 5.2	3D Images Multiple Values per pixel Hyperspectral Imaging Image Formation Acquiring Images Traditional Imaging: Model Prent views on image Analysis Image Analysis: Experimentalist Image Analysis: Computer Vision Approaches Image Analysis: Deep Learning Approach	35 37 39 41 42 44 47 48 48
5	4.4 4.5 4.6 4.7 4.8 4.9 Diffe 5.1 5.2 5.3 5.4	3D Images Multiple Values per pixel Hyperspectral Imaging Image Formation Acquiring Images Traditional Imaging: Model Prent views on image Analysis Image Analysis: Experimentalist Image Analysis: Computer Vision Approaches Image Analysis: Deep Learning Approach Summary analysis approaches	35 37 39 41 42 44 47 48 48 49
	4.4 4.5 4.6 4.7 4.8 4.9 Diffe 5.1 5.2 5.3 5.4	3D Images Multiple Values per pixel Hyperspectral Imaging Image Formation Acquiring Images Traditional Imaging: Model rent views on image Analysis Image Analysis: Experimentalist Image Analysis: Computer Vision Approaches Image Analysis: Deep Learning Approach Summary analysis approaches	35 37 39 41 42 44 47 48 48 49 49
	4.4 4.5 4.6 4.7 4.8 4.9 Diffe 5.1 5.2 5.3 5.4 On S	3D Images Multiple Values per pixel Hyperspectral Imaging Image Formation Acquiring Images Traditional Imaging: Model rent views on image Analysis Image Analysis: Experimentalist Image Analysis: Computer Vision Approaches Image Analysis: Deep Learning Approach Summary analysis approaches Science What is the purpose?	35 37 39 41 42 44 47 48 49 49

6.3	Important questions	52
6.4		56
6.5	Intensity gradients	56
6.6	Selecting the right tool for your task	57
6.7	Reproducibility vs. Repeatability	59
6.8	Reproducibility vs. Repeatability	60
6.9	How can we keep track of everything for ourselves and others?	60
Worl	kflows for image analysis	61
7.1	Computing has changed: Parallel	61
7.2		62
7.3		63
7.4		64
7.5		
7.6		
Sum	mary	75
8.1		75
	6.4 6.5 6.6 6.7 6.8 6.9 Wor 7.1 7.2 7.3 7.4 7.5 7.6 Sum	6.4 Why quantitative? 6.5 Intensity gradients 6.6 Selecting the right tool for your task 6.7 Reproducibility vs. Repeatability 6.8 Reproducibility vs. Repeatability 6.9 How can we keep track of everything for ourselves and others? Workflows for image analysis 7.1 Computing has changed: Parallel 7.2 Computing has changed: Cloud 7.3 Workflow analysis 7.4 Using flow charts / workflows 7.5 Directed Acyclical Graphs (DAG) 7.6 Deep Learning Summary

Quantitative Big Imaging ETHZ: 227-0966-00L

CONTENTS 1

2 CONTENTS

CHAPTER

ONE

TODAYS LECTURE

- · About the course
- Motivating the use of quantitive methods in imaging
- What is an image?
- Where do images come from?
- · Science and Reproducibility
- · Workflows

1.1 We need some python modules

Python is a modular scripting language with limited functionality. Features are added through modules that are imported. These are the modules that are needed for this lecture. Please run this cell before you start using the notebook.

```
import numpy as np
import pandas as pd
import matplotlib.pyplot as plt
%matplotlib inline

from skimage.io import imread
from scipy.ndimage import convolve
from skimage.morphology import disk
from skimage.transform import resize
from itertools import product
import os
from io import StringIO
```

ABOUT THE COURSE

- Who are we?
- · Who are you?
- What is expected?
- Why does this class exist?
- Collection
- Changing computing (Parallel / Cloud)
- · Course outline

2.1 Who are we?

Anders Kaestner

You will hear me a lot during this course. I am the lecturer and I will also support you with problems during the exercises.

- Beamline scientist at the ICON Beamline at the SINQ (Neutron Source) at Paul Scherrer Institute
 - Lecturer at ETH Zurich
- Algorithm developer Varian Medical Systems, Baden-Daettwil
- Post Doc at ETH Zurich, Inst for Terrestial Ecology
- PhD at Chalmers Institute of Technology, Sweden, Signal processing

anders.kaestner@psi.ch

Stefano van Gogh

Will help you during the exercise sessions.

- PhD Student in the X-Ray Microscopy Group at ETH Zurich and Swiss Light Source at Paul Scherrer Institute
- · Teaching assistant

stefano.van-gogh@psi.ch

2.2 Who are you?

This course is targeting a wide range of students with different levels of experience. In the table you'll see were students came from in previos years. Some have a technical background others are merely producing images in the line of their project and have never seen much more than photoshop and similar programs for processing image data. Using some kind of programming is nescessary to perform quantitative image analysis on large data sets. A single or a few images can easily be handled with interactive software, but taking it beyond that is hard without writing some lines of code.

Now, some of you have little to no programming experience while others have been programming since they got their first computer in the hand.

A wide spectrum of backgrounds	A wide range of skills
Biomedical Engineers	
Physicists	I think I've heard of python before
Chemists	
Art History Researchers	
Mechanical Engineers	I write template C++ code and hand optimize it afterwards
and Computer Scientists	

2.3 So how will this ever work?

Now you maybe start to get worried! The purpose of this course is not to teach you programming but rather to provide you with a bag full recipes that you can use in your projects. Most of these recipes are just a list of the commands from different python moduls that you need to perform your analysis. A side-effect will probably be that you learn one or two programming tricks on the way.

In the lectures, there will be small code pieces on the slides. Some of these are there to illustrate how an operation works, while other parts are there for the nice presentation of the results (this is mostly the second half of the code cell). Presenting the results is important. In the end, you want to show your results to the scinetific community. So even though the plotting clutters the slide, there is something to learn there as well.

Adaptive assignments

- · Conceptual, graphical assignments with practical examples
 - Emphasis on chosing correct steps and understanding workflow
- Opportunities to create custom implementations, and perform more complicated analysis on larger datasets if interested
 - Emphasis on performance, customizing analysis, and scalability

2.4 Course Expectations

The practical part of the course has two parts. None of these are mandatory, but they will help you to better understand use the material you have learnt in the lectures.

Exercises		
Usually 1 set per lecture		
Optional (but recommended!)		
Easy - jupyter notebooks are prepared for the exercises		
Advanced - Writing Python, Java, C++,		

The exercises are prepared in a way that you learn step by step what you need to do and guids you through the problems. We will be using jupyter notebooks for the lectures. This is a very common way to work with image data these days.

Science project		
Optional (but strongly recommended)		
Applying Techniques to answer scientific question!		
Ideally use on a topic relevant for your current project, thesis, or personal activities		
or choose from one of ours (will be online, soon)		
Present approach, analysis, and results		

In the optional science projects you will have to opportunity to test what you have learned during the course on real problems. This is the place for your creativity.

2.5 Projects

- · A small image processing project
- Can be related to you Master or PhD project
- You will get input and ideas for your own projects
- · You will get hands on experience on the techniques you learn here
- Can be used as discussion base for your exam

2.6 Course Overview

Topic	Date	Title	Description
Introduc-	25th	Introduction and Workflows	Basic overview of the course, introduc-
tion	February		tion to
Data 4th March		Image Enhancement	Overview of what techniques are avail-
			able for
	11th	Ground Truth: Building and Augmenting	Examples of large datasets, how they
	March	Datasets	were buil
Segmenta-	18th	Basic Segmentation, Discrete Binary	How to convert images into structures,
tion	tion March Structures		startin
	25th	Advanced Segmentation	More advanced techniques for extracting
	March		struct
	1st April	Supervised Problems and Segmentation	More advanced techniques for extracting
			struct
	8th April	Easter break	Search for eggs
Analysis	15th April	Analyzing Single Objects Shape and	The analysis and characterization of sin-
Analysis	13ui Apiii	Analyzing Single Objects, Shape, and Texture	gle st
	22th April	Analyzing Complex Objects and Distri-	What techniques are available to analyze
	ZZui Apin	butions	more
	29th April	Dynamic Experiments	Performing tracking and registration in
	Z9tii Aprii	Dynamic Experiments	dynami
Big Imag-	6th May	Imaging with multiple modalities	Combining information from different
ing	our way	imaging with matupic modulities	sources
mg .	13th May	Ascension	Enjoy a lovely early summers day
	13ui iiuy	1 isochistori	Enjoy a lovely early summers day
	20th May	Scaling Up / Big Data	Performing large scale analyses on clus-
			ters
Wrapping up	27th May	Project Presentations	You present your projects

2.7 Today's Reading Material

- Some book on image processing with python (to be updated)
- Cloud Computing
- The Case for Energy-Proportional Computing Luiz André Barroso, Urs Hölzle, IEEE Computer, December 2007
- Concurrency
- · Reproducibility
- Trouble at the lab Scientists like to think of science as self-correcting. To an alarming degree, it is not
- Why is reproducible research important? The Real Reason Reproducible Research is Important
- Science Code Manifesto
- Reproducible Research Class @ Johns Hopkins University

2.8 Literature / Useful References

These are books that are useful in many of the lectures. In particular the Image processing hand book by John Russ shows you an overview of typical image processing techniques.

- John C. Russ, "The Image Processing Handbook", (Boca Raton, CRC Press)
 - Available online within domain ethz.ch (or proxy.ethz.ch / public VPN)
- Jean Claude, Morphometry with R
 - Online through ETHZ

MOTIVATION - YOU HAVE DATA!

3.1 Imaging experiments produce a lot of data

Working with imaging techniques you will get a lot of images that shows the sample in the eye of the technique you are using. The experiment were you acquire these images is only a small fraction of the complete workflow from idea to the final scientific publiction. The amout of data can also be overwhelming for many scientist with the consequence that the data is never analyzed properly, and then also not published in the way it really deserves.









Gigabytes...
...or even
terabytes of data

Fig. 3.1: A typical imaging experiment produces large amounts of data.

3.2 Motivation - how to proceed?

Now is the question how to proceed towards a working analysis workflow that results in repeatable analyses for your data.

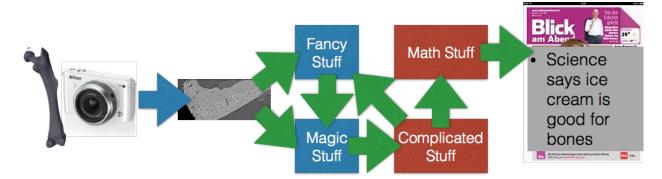


Fig. 3.2: A crazy unstructured and unclear work flow to analyze images from your experiment.

- To understand what, why and how from the moment an image is produced until it is finished (published, used in a report, ...)
- To learn how to go from one analysis on one image to 10, 100, or 1000 images (without working 10, 100, or 1000X harder)

3.3 High acquisition rates

The trend in imaging is that experimentalist want to follow faster and faster processes. This wish can be supported the technical development of new detectors that provide very high acqisition rates. Here, we can also see that some cameras are able to produce more data than is uploaded per day on facebook and instagram!

- Detectors are getting bigger and faster constantly
- · Todays detectors are really fast
 - 2560 x 2160 images @ 1500+ times a second = 8GB/s
- Matlab / Avizo / Python / ... are saturated after 60 seconds

Many of the analysis platforms are already overwhelmed with handling the data rates produced by typical detector systems at imaging instrument. This restriction is partly due to hardware limitations. The memory is to small, hard drives are not sufficiently fast. The other side of the problem is that these tools are not prepared to work with large data streams.

- A single camera
 - More information per day than Facebook
 - Three times as many images per second as Instagram

3.4 Different sources of images

Images are produced by many different detectors and in some cases they are even the output from simulations. In the next sections we see some different imaging modalities and the data rates they produce.

3.4.1 X-Ray

X-ray imaging at syncrotron light sources produces very high frame rates thanks to the high brilliance of the source. Here are some examples of data rates from some instruments.

- SRXTM images at (>1000fps) \rightarrow 8GB/s
- cSAXS diffraction patterns at 30GB/s
- Nanoscopium Beamline, 10TB/day, 10-500GB file sizes

3.4.2 Optical

Optical imaging methods are more modest than the X-ray techniques, but still they produce data in the order of some hundred Mb per second.

- Light-sheet microscopy (see talk of Jeremy Freeman) produces images → 500MB/s
- High-speed confocal images at (>200fps) \rightarrow 78Mb/s

3.4.3 Personal

Finally, we also take a look at cameras on the consumer market and see that these devices also produce relatively high data rates. This data must mostly be handled by normal household computers, which can be a challenging task...

- GoPro 4 Black 60MB/s (3840 x 2160 x 30fps) for \$600
- fps1000 400MB/s (640 x 480 x 840 fps) for \$400

3.5 The experiment life cycle

Now we have seen that there is a wish to obtain data at high rates and that there are technological solutions to provide this. The remaining part to develop is the post processing.

- 1. **Experimental Design** finding the right technique, picking the right dyes and samples has stayed relatively consistent, better techniques lead to more demanding scientists.
- 2. **Measurements** the actual acquisition speed of the data has increased wildly due to better detectors, parallel measurement, and new higher intensity sources
- 3. **Management** storing, backing up, setting up databases, these processes have become easier and more automated as data magnitudes have increased
- 4. **Post Processing** this portion has is the most time-consuming and difficult and has seen minimal improvements over the last years

The post processing is the least trivial part to generalize. The initial steps are often possible to generalize as these are operations that all types of imaging experiments need to go through. When it comes to the experiment specific

analysis the degree of generalization decreases and the scientists are left to develop their own procedure to extract the quantitative information from the images.

3.5.1 How is time used during the experiment life cycle?

With the development of faster acquisision systems there has been a change in the ratio between

- Exeperiment design and preparation
- Measurements
- · Data management
- · and post processing

over the years. This in particular the case for X-ray imaging where the flux is high and the acquisition is limited by the detector technology. In other modalities, where the measurement is flux limited we see a different distribution.

What also increases the post processing time is that the experiments have become more complicated over the years. Twenty years ago, it was sufficient to show qualitative information a beautify volume rendering or a movie of the sample. Meanwhile, it has become a requirement that you provide quantitative results from the images.

3.6 Handling masses of images

3.6.1 So... how much is a TB, really?

We have been talking about different data amounts of MB, GB, and TB. But, what does that really mean in reality? Let us explore what is a TB.

If **you** looked at one image with 1000 x 1000 pixels (1 Mpixels)

Here we create one image with 1000x1000 pixels with random values form a uniform distribution [0,1] and show it.

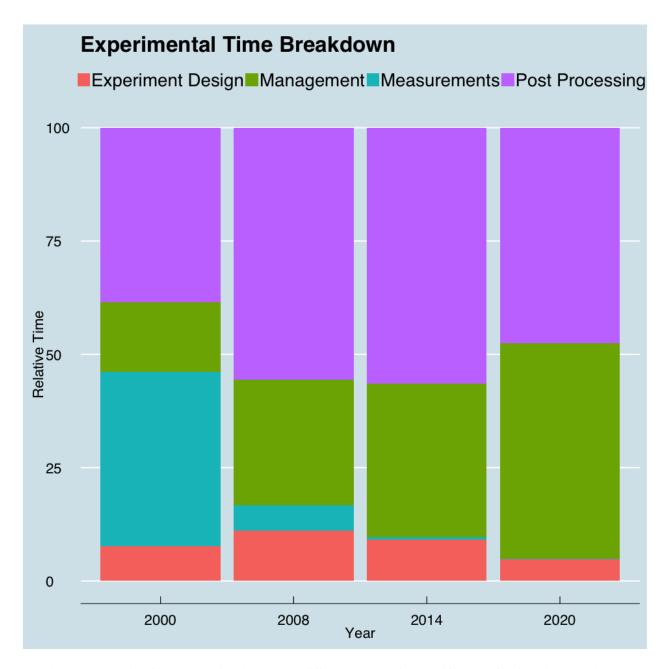
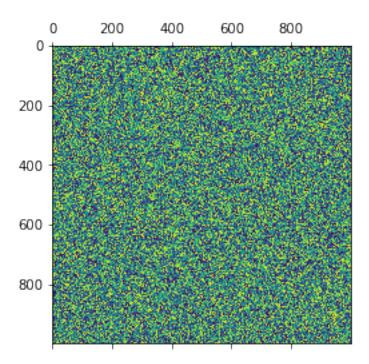


Fig. 3.3: The ratio of how much time is spent on different tasks during the lifecycle of an imaging experiment.



every second, it would take you

```
# assuming 16 bit images and a 'metric' terabyte
OneTB = 1e12
ImageSize = 1000*1000*16/8
hour = 60*60

time_per_tb = OneTB/ImageSize/hour
print("{0:0.1f} hours to view a terabyte".format(time_per_tb))
```

```
138.9 hours to view a terabyte
```

3.6.2 Overwhelmed scientist

Providing quantitative statements about image data is often very hard. You can may manage to do it on a single images like the bone image below.

You would like to know:

- Count how many cells are in the bone slice.
- Ignore the ones that are 'too big' or shaped 'strangely'.
- Are there more on the right side or left side?
- Are the ones on the right or left bigger, top or bottom?



Fig. 3.4: A slice image show bone cells.

3.6.3 More overwhelmed scientist

Statistical analysis requires that you study many samples and not just a single one. The samples are also objects which requires 3D data instead of a single 2D slice.

Many samples are needed:

- Do it all over again for 96 more samples
- This time in 3D with 2000 slices instead of just one!

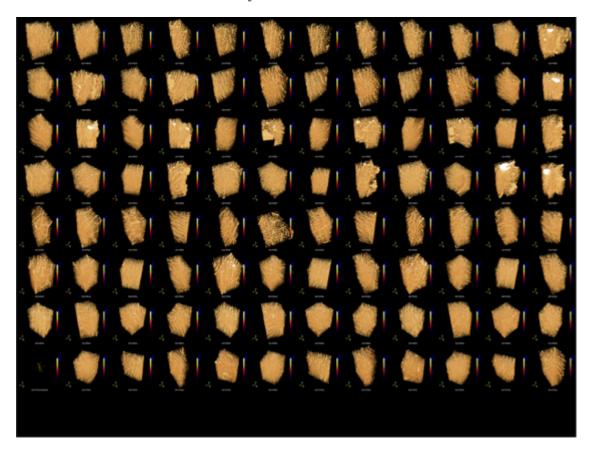


Fig. 3.5: A collection of 96 volume images from different bone samples.

Working with multiple 3D images is not feasible anymore to do manually. We need some kind of automated process to perform the analysis.

3.6.4 Bring on the pain

The 96 samples only represented one of our cases in the study. Now, if we want to study different ages, healthy/diseased, etc, we need to add a sample batch for each case. Maybe we even need to increase the number of samples in each test group. With all these variations, we can easily end up in a thousand samples to analyze.

Great variations in the population

- Now again with 1090 samples!
- How to measure?
- How to analyze?

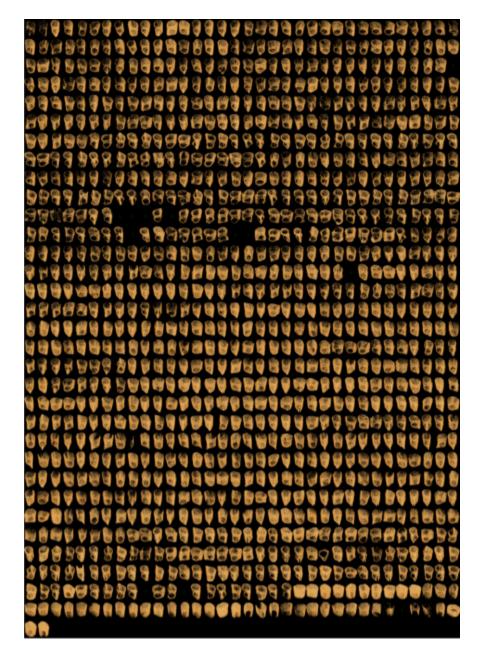


Fig. 3.6: A collection of 1090 bone samples. This is a massive task to analyze!

With so many samples we stand in front of a logistic problem to measure the data and once the data is there we have to analyze it. As a first step, we have to specify how to analyze these images to obtain results that may or may not support a hypothesis.

3.6.5 It gets better

The metrics we specified in the previous example are easy to observe and also to measure. They are direct measurements of pixels and positions. What if we now want to make more complicated inquiries even. Now how do we categorize the images or collections of features using soft metrics?

- Those metrics were quantitative and could be easily visually extracted from the images
- What happens if you have softer metrics
 - How aligned are these cells?
 - Is the group on the left more or less aligned than the right?
 - errr?

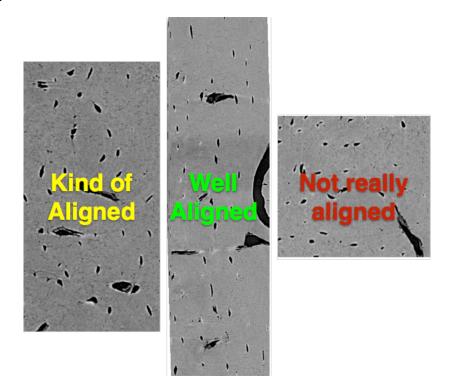


Fig. 3.7: Close-up on different bone segments. How aligned are the cells in these images?

3.6.6 Dynamic Information

Many experiments are on top of the spatial dimensions also studies over time. This brings us 4D data sets to analyze. How are we supposed to handle this? Looking at the movie we

- How many bubbles are here?
- How fast are they moving?
- Do they all move the same speed?
- Do bigger bubbles move faster?
- Do bubbles near the edge move slower?
- Are they rearranging?

CHAPTER

FOUR

IMAGES

4.1 An introduction to images

4.1.1 What is an image?

A very abstract definition:

- A pairing between spatial information (position)
- and some other kind of information (value).

In most cases this is a 2- or 3-dimensional position (x,y,z coordinates) and a numeric value (intensity)

4.1.2 Image sampling

The world is	The computer needs
Continuous	Discrete levels
No boundaries	Limited extent

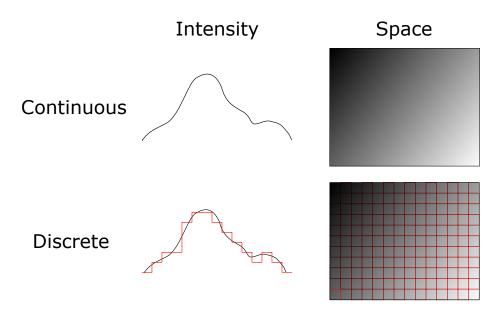
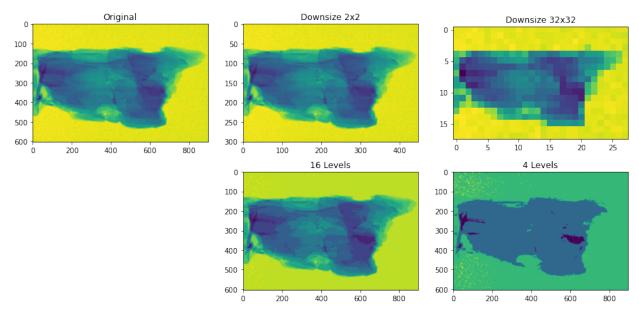


Fig. 4.1: The real world is sampled into discrete images with limited extent.

4.1.3 What does sampling mean

```
img=np.load('../../common/data/wood.npy');
plt.figure(figsize=[15,7])
plt.subplot(2,3,1); plt.imshow(img); plt.title('Original')
downsize = 2; plt.subplot(2,3,2); plt.imshow(resize(img,(img.shape[0] // downsize,__
img.shape[1] // downsize), anti_aliasing=False)); plt.title('Downsize {0}x{0}'.
img.shape[1] // downsize))
downsize = 32; plt.subplot(2,3,3); plt.imshow(resize(img,(img.shape[0] // downsize,__
img.shape[1] // downsize), anti_aliasing=False)); plt.title('Downsize {0}x{0}'.
img.shape[1] // downsize))
levels = 16; plt.subplot(2,3,5); plt.imshow(np.floor(img*levels)); plt.title('{0}_
Levels'.format(levels));
levels = 4; plt.subplot(2,3,6); plt.imshow(np.floor(img*levels)); plt.title('{0}_
Levels'.format(levels));
```

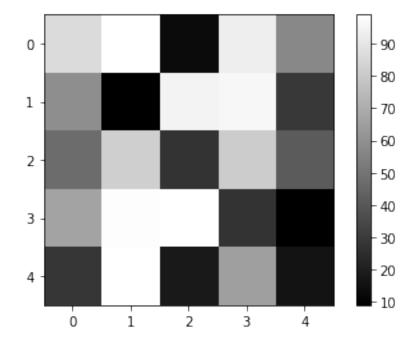


4.2 Let's create a small image

```
Х
       Intensity
  V
0
   0
               53
1
    0
               14
2
    Λ
               82
               78
3
    Ω
4
   0
                2
```

24 Chapter 4. Images

```
import matplotlib.pyplot as plt
plt.imshow(basic_image, cmap = 'gray')
plt.colorbar();
```



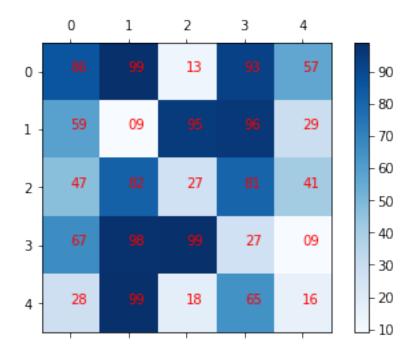
4.3 2D Intensity Images

The next step is to apply a color map (also called lookup table, LUT) to the image

- so it is a bit more exciting
- some features are easier to detect Rogowitz et al. 1996

```
fig, ax1 = plt.subplots(1,1)
plot_image = ax1.matshow(basic_image, cmap = 'Blues')
plt.colorbar(plot_image)

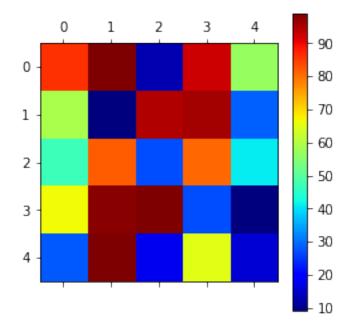
for _, c_row in image_df.iterrows():
    ax1.text(c_row['x'], c_row['y'], s = '%02d' % c_row['Intensity'], fontdict = __
    dict(color = 'r'))
```



4.3.1 Different colormaps

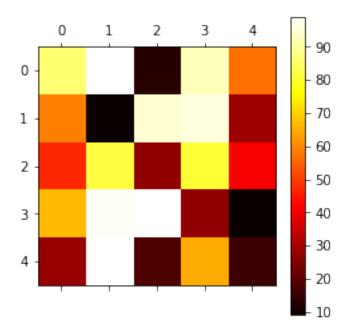
Color maps can be arbitrarily defined based on how we would like to visualize the information in the image

```
plot_image = plt.matshow(basic_image, cmap = 'jet')
plt.colorbar(plot_image);
```



```
plot_image = plt.matshow(basic_image, cmap = 'hot')
plt.colorbar(plot_image);
```

26 Chapter 4. Images



4.3.2 Lookup Tables

Formally a color map is lookup table or a function which $f(Intensity) \rightarrow Color$

Matplotlib's color maps

```
import numpy as np
import matplotlib as mpl
import matplotlib.pyplot as plt
from matplotlib import cm
#from colorspacious import cspace_converter
from collections import OrderedDict
cmaps = OrderedDict()
cmaps['Perceptually Uniform Sequential'] = [
             'viridis', 'plasma', 'inferno', 'magma', 'cividis']
cmaps['Sequential'] = [
             'Greys', 'Purples', 'Blues', 'Greens', 'Oranges', 'Reds',
             'YlorBr', 'YlorRd', 'OrRd', 'PuRd', 'RdPu', 'BuPu',
             'GnBu', 'PuBu', 'YlGnBu', 'PuBuGn', 'BuGn', 'YlGn']
cmaps['Sequential (2)'] = [
             'binary', 'gist_yarg', 'gist_gray', 'gray', 'bone', 'pink', 'spring', 'summer', 'autumn', 'winter', 'cool', 'Wistia',
             'hot', 'afmhot', 'gist_heat', 'copper']
cmaps['Diverging'] = [
             'PiYG', 'PRGn', 'BrBG', 'PuOr', 'RdGy', 'RdBu',
             'RdYlBu', 'RdYlGn', 'Spectral', 'coolwarm', 'bwr', 'seismic']
cmaps['Cyclic'] = ['twilight', 'twilight_shifted', 'hsv']
```

(continues on next page)

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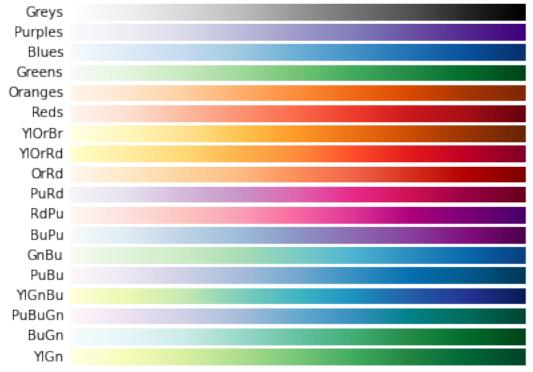
```
cmaps['Qualitative'] = ['Pastel1', 'Pastel2', 'Paired', 'Accent',
                        'Dark2', 'Set1', 'Set2', 'Set3',
                        'tab10', 'tab20', 'tab20b', 'tab20c']
cmaps['Miscellaneous'] = [
            'flag', 'prism', 'ocean', 'gist_earth', 'terrain', 'gist_stern',
            'gnuplot', 'gnuplot2', 'CMRmap', 'cubehelix', 'brg',
            'gist_rainbow', 'rainbow', 'jet', 'nipy_spectral', 'gist_ncar']
nrows = max(len(cmap_list) for cmap_category, cmap_list in cmaps.items())
gradient = np.linspace(0, 1, 256)
gradient = np.vstack((gradient, gradient))
def plot_color_gradients(cmap_category, cmap_list, nrows):
    fig, axes = plt.subplots(nrows=nrows)
    fig.subplots_adjust(top=0.95, bottom=0.01, left=0.2, right=0.99)
   axes[0].set_title(cmap_category + ' colormaps', fontsize=14)
    for ax, name in zip(axes, cmap_list):
       ax.imshow(gradient, aspect='auto', cmap=plt.get_cmap(name))
       pos = list(ax.get_position().bounds)
       x_{text} = pos[0] - 0.01
       y_{text} = pos[1] + pos[3]/2.
       fig.text(x_text, y_text, name, va='center', ha='right', fontsize=10)
    # Turn off *all* ticks & spines, not just the ones with colormaps.
   for ax in axes:
       ax.set_axis_off()
for cmap_category, cmap_list in cmaps.items():
   plot_color_gradients(cmap_category, cmap_list, nrows)
```

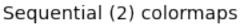
28 Chapter 4. Images

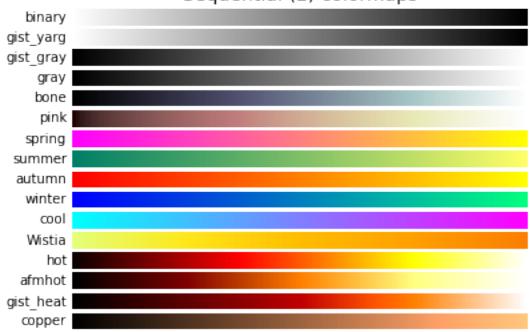




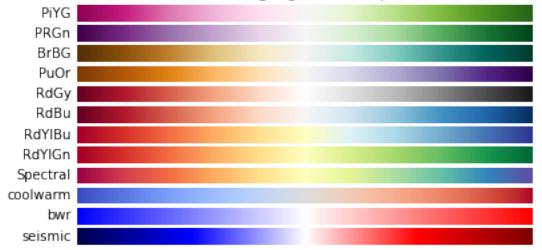
Sequential colormaps







Diverging colormaps

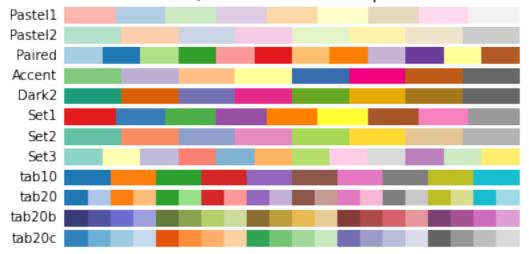


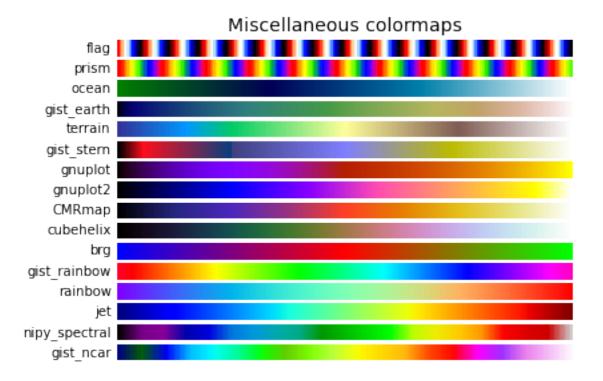
30 Chapter 4. Images





Qualitative colormaps

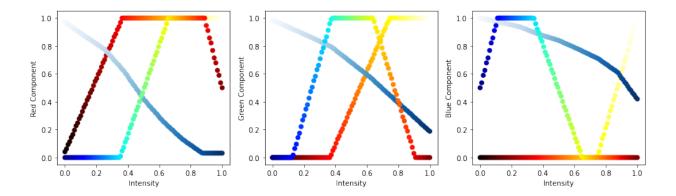




4.3.3 How are the colors combined

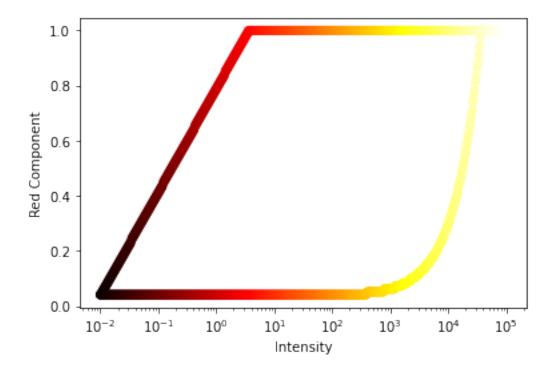
```
%matplotlib inline
import matplotlib.pyplot as plt
import numpy as np
xlin = np.linspace(0, 1, 100)
colors = ['Red','Green','Blue']
plt.figure(figsize=[15,4])
for i in np.arange(0,3):
   plt.subplot(1,3,i+1)
   plt.scatter(xlin,
           plt.cm.hot(xlin)[:,i],
           c = plt.cm.hot(xlin),label="hot")
   plt.scatter(xlin,
           plt.cm.Blues(xlin)[:,i],
           c = plt.cm.Blues(xlin), label="blues")
   plt.scatter(xlin,
           plt.cm.jet(xlin)[:,i],
           c = plt.cm.jet(xlin),label='jet')
   plt.xlabel('Intensity');
   plt.ylabel('{0} Component'.format(colors[i]));
```

32 Chapter 4. Images



4.3.4 Applied LUTs

These transformations can also be non-linear as is the case of the graph below where the mapping between the intensity and the color is a log relationship meaning the the difference between the lower values is much clearer than the higher ones



4.3.5 LUTs on real images

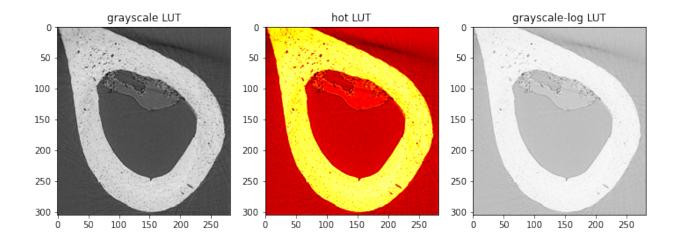
On a real image the difference is even clearer

```
%matplotlib inline
import matplotlib.pyplot as plt
from skimage.io import imread
fig, (ax1, ax2, ax3) = plt.subplots(1,3, figsize = (12, 4))
in_img = imread('figures/bone-section.png')[:,:,0].astype(np.float32)
ax1.imshow(in_img, cmap = 'gray');
ax1.set_title('grayscale LUT');

ax2.imshow(in_img, cmap = 'hot');
ax2.set_title('hot LUT');

ax3.imshow(np.log2(in_img+1), cmap = 'gray');
ax3.set_title('grayscale-log LUT');
```

34 Chapter 4. Images



4.4 3D Images

For a 3D image, the position or spatial component has a 3rd dimension (z if it is a spatial, or t if it is a movie)

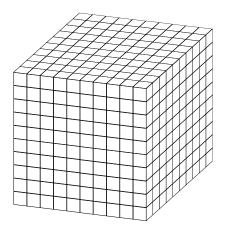


Fig. 4.2: Three-dimensional data can be a volume in space.

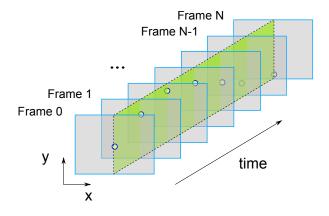


Fig. 4.3: A movie can also be seen as a three-dimensional image.

4.4. 3D Images 35

4.4.1 A 3D image as array

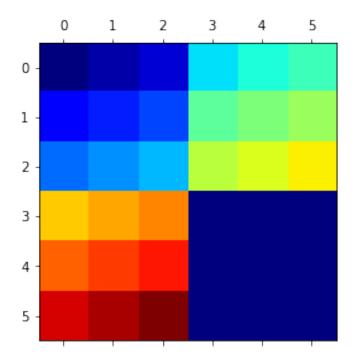
```
import numpy as np
vol_image = np.arange(27).reshape((3,3,3))
print(vol_image)
```

4.4.2 Showing 2D slices from volume

This can then be rearranged from a table form into an array form and displayed as a series of slices

```
%matplotlib inline
import matplotlib.pyplot as plt
from skimage.util import montage as montage2d
print(montage2d(vol_image, fill = 0))
plt.matshow(montage2d(vol_image, fill = 0), cmap = 'jet');
```

36 Chapter 4. Images



4.5 Multiple Values per pixel

In the images thus far, we have had one value per position, but there is no reason there cannot be multiple values. In fact this is what color images are (red, green, and blue) values and even 4 channels with transparency (alpha) as a different. For clarity we call the **dimensionality** of the image the number of dimensions in the spatial position, and the **depth** the number in the value.

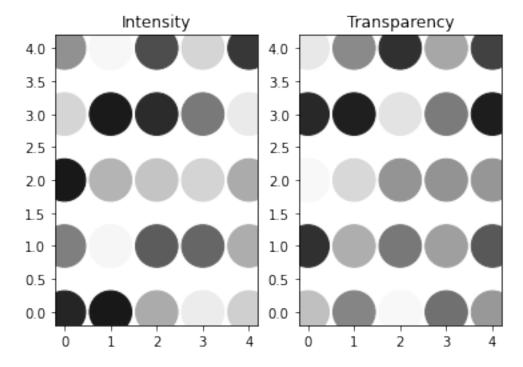
```
base_df = pd.DataFrame([dict(x = x, y = y) for x,y in product(range(5), range(5))])
base_df['Intensity'] = np.random.uniform(0, 1, 25)
base_df['Transparency'] = np.random.uniform(0, 1, 25)
base_df.head(5)
```

```
Transparency
         Intensity
   0
          0.144754
                         0.750454
      0
   0
      1
                         0.189002
1
          0.496202
   0
      2
          0.094933
                         0.968853
      3
          0.833134
                         0.158436
          0.566797
                         0.909068
```

This can then be rearranged from a table form into an array form and displayed as a series of slices

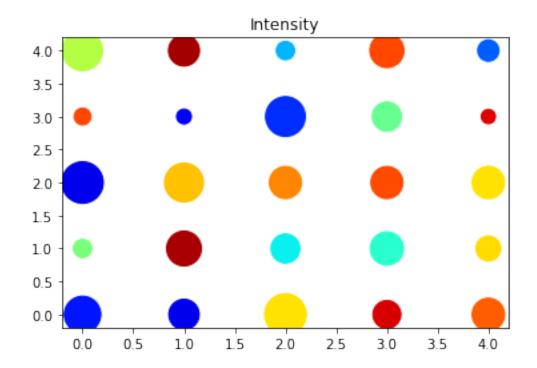
4.5.1 Display multi-valued pixels separately

The most straight forward way to display multiple pixel values is to display each value separately. This method is, however, mostly not very suitable as the values often are related in some sense. Therefore it is recommended to combine the values in the same plot.



In this example we combined two values use one value to control the colormap and the other to control the size of the dots. How you combine the data is related to the to of data you want to combine. If the values are components of a vector it makes more sense to show arrows of different length and direction, etc.

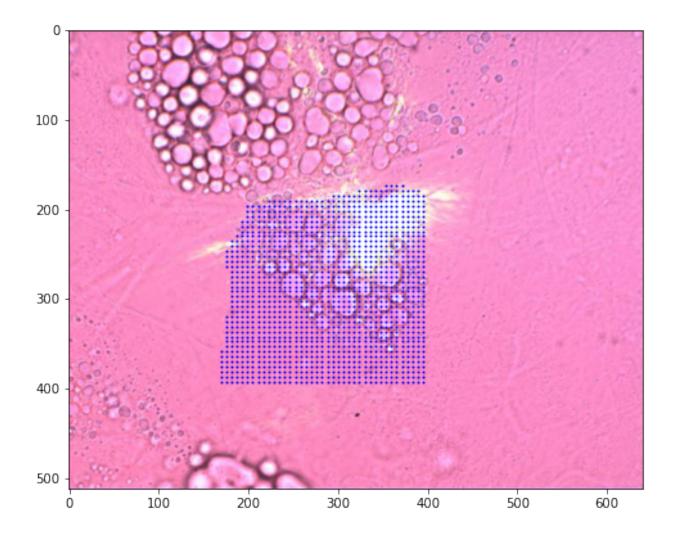
38 Chapter 4. Images



4.6 Hyperspectral Imaging

At each point in the image (black dot), instead of having just a single value, there is an entire spectrum. A selected group of these (red dots) are shown to illustrate the variations inside the sample. While certainly much more complicated, this still constitutes and image and requires the same sort of techniques to process correctly.

```
%matplotlib inline
import matplotlib.pyplot as plt
import pandas as pd
from skimage.io import imread
import os
raw_img = imread('../../common/data/raw.jpg')
im_pos = pd.read_csv('../../common/data/impos.csv', header = None)
im_pos.columns = ['x', 'y']
fig, ax1 = plt.subplots(1,1, figsize = (8, 8));
ax1.imshow(raw_img);
ax1.scatter(im_pos['x'], im_pos['y'], s = 1, c = 'blue');
```



4.6.1 Looking at the pixel values

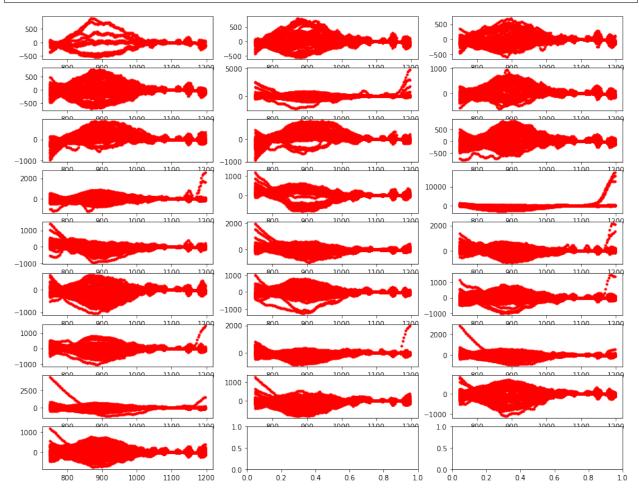
Each pixel of this data set is represented by a full spectrum. This means that we have both wave numbers and intensity values. In many cases, the wavenumbers are the same for all pixels, which makes it possible to reduce the redundancy of the wave number vector using a single 1D array to represent this information.

```
full_df = pd.read_csv('../../common/data/full_img.csv').query('wavenum<1200')
print(full_df.shape[0], 'rows')
full_df.head(5)</pre>
```

210750 rows

```
Х
               wavenum
168.95
        358.8
                   750
                        527.571102
168.95
        358.8
                   753
                        459.778584
        358.8
                   756
                        406.337255
168.95
        358.8
                   759
                        341.858123
                   762 246.645673
168.95
       358.8
```

40 Chapter 4. Images



4.7 Image Formation

The image formation process is the process to use some kind of excitation or impulse probe a sample. This requires the interaction of four parts.

- Impulses Light, X-Rays, Electrons, A sharp point, Magnetic field, Sound wave
- Characteristics Electron Shell Levels, Electron Density, Phonons energy levels, Electronic, Spins, Molecular mobility
- Response Absorption, Reflection, Phase Shift, Scattering, Emission
- Detection Your eye, Light sensitive film, CCD / CMOS, Scintillator, Transducer

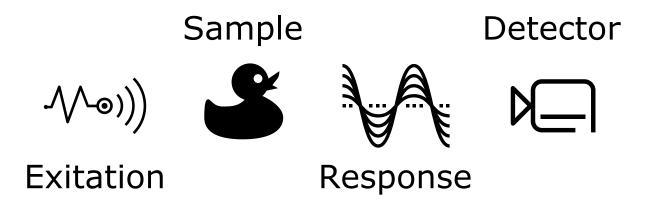


Fig. 4.4: The parts involved in the image formation process probing a sample.

4.7.1 Where do images come from?

Modality	Impulse	Characteristic	Response	Detection
Light Mi-	White Light	Electronic interactions	Absorption	Film, Camera
croscopy				
Phase Contrast	Coherent light	Electron Density (Index of	Phase Shift	Phase stepping, hologra-
		Refraction)		phy, Zernike
Confocal	Laser Light	Electronic Transition in Flu-	Absorption and	Pinhole in focal plane,
Microscopy		orescence Molecule	reemission	scanning detection
X-Ray Radiog-	X-Ray light	Photo effect and Compton	Absorption and	Scintillator, microscope,
raphy		scattering	scattering	camera
Neutron Radio-	Neutrons	Interaction with nucleus	Scattering and	Scintillator, optics, cam-
graphy			absorption	era
Ultrasound	High frequency	Molecular mobility	Reflection and	Transducer
	sound waves		Scattering	
MRI	Radio-frequency	Unmatched Hydrogen spins	Absorption and	RF coils to detect
	EM		reemission	
Atomic Force	Sharp Point	Surface Contact	Contact, Repul-	Deflection of a tiny mir-
Microscopy			sion	ror

4.8 Acquiring Images

4.8.1 Traditional / Direct imaging

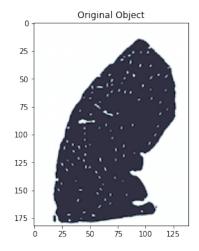
- Visible images produced or can be easily made visible
- Optical imaging, microscopy

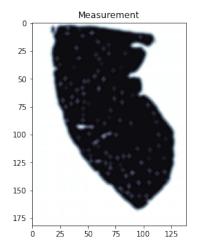
```
bone_img = imread('figures/tiny-bone.png').astype(np.float32)
# simulate measured image
conv_kern = np.pad(disk(2), 1, 'constant', constant_values = 0)
meas_img = convolve(bone_img[::-1], conv_kern)
# run deconvolution
dekern = np.fft.ifft2(1/np.fft.fft2(conv_kern))
```

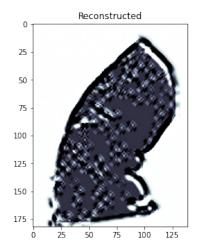
(continues on next page)

42 Chapter 4. Images

(continued from previous page)



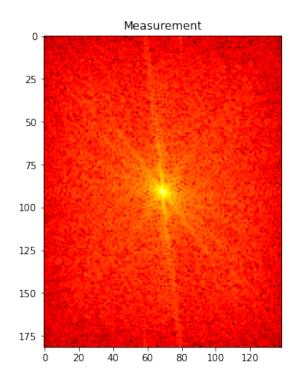


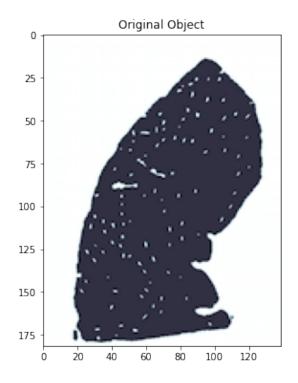


4.8.2 Indirect / Computational imaging

- Recorded information does not resemble object
- Response must be transformed (usually computationally) to produce an image

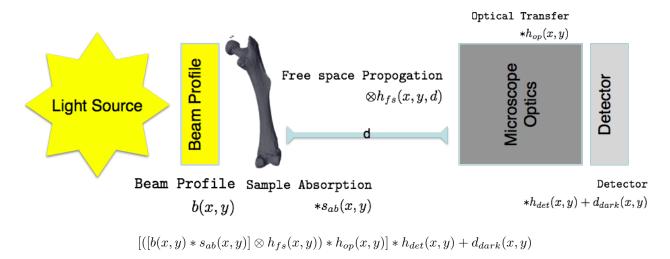
1.146423838816545 6.61125552595089 3.3563935033662253





4.8.3 Traditional Imaging

4.9 Traditional Imaging: Model



 s_{ab} is the only information you are really interested in, so it is important to remove or correct for the other components For color (non-monochromatic) images the problem becomes even more complicated $\int_0^\infty \left[([b(x,y,\lambda)*s_{ab}(x,y,\lambda)]\otimes h_{fs}(x,y,\lambda))*h_{op}(x,y,\lambda)\right]*h_{det}(x,y,\lambda)\mathrm{d}\lambda + d_{dark}(x,y)$

44 Chapter 4. Images

4.9.1 Indirect Imaging (Computational Imaging)

With indirect imaging you make acquisitions in a form that don't represent the information you want to have. It is needed to perform a numeric transformation to obtain images in observation space.

Some examples are:

- Tomography through projections
- · Microlenses Light-field photography
- Diffraction patterns
- Hyperspectral imaging with Raman, IR, CARS
- Surface Topography with cantilevers (AFM)
- Tomography through projections
- Microlenses Light-field photography
- Diffraction patterns
- Hyperspectral imaging with Raman, IR, CARS
- Surface Topography with cantilevers (AFM)

46 Chapter 4. Images

DIFFERENT VIEWS ON IMAGE ANALYSIS

Image analysis is a complex task and there are many ways to reach the quantitative results from the images.

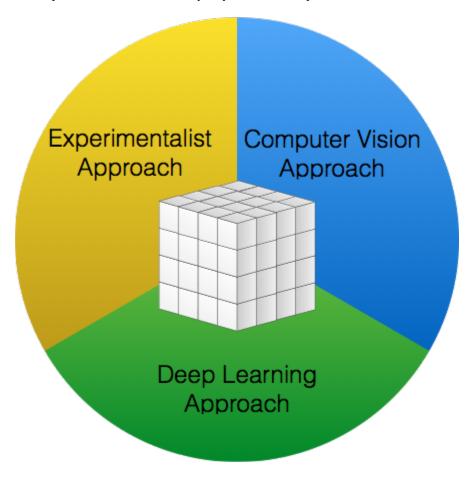


Fig. 5.1: Your background often decides how you approach an image analysis problem.

We can make two initial statements:

- An image is a bucket of pixels.
- · How you choose to turn it into useful information is strongly dependent on your background

5.1 Image Analysis: Experimentalist

The experimentalist looks with a problem driven concept on the analysis task. It is often a top down approach aiming at solving the specific problem at hand. The solution is often reality driven and aims at finding models explaining the information presented in the images.

Typical task the experimentalist tries to solve are very practical and specific like counting cells in the image or to measure the porosity of the sample.

5.1.1 Characteristics

- · Problem-driven
- Top-down
- · Reality Model-based

5.1.2 Examples

- · cell counting
- porosity

5.2 Image Analysis: Computer Vision Approaches

The computer vision/signal processing scientist works to develop methods to solve a class of image processing problem. The approach is based on abstract features found in the image. The models are based on features and noise found in the images. The systematic appoach is even based on engineered image features to better test and evaluate the developed methods.

The computer vision approach is typical looking to detect features like edges, structures, and also complicated features like faces.

5.2.1 Characteristics

- · Method-driven
- · Feature-based
- Image Model-based
- Engineer features for solving problems

5.2.2 Examples

- Edge detection
- · Face detection

5.3 Image Analysis: Deep Learning Approach

Finally, the deep learning approach is data driven and inspired by the way nature solves the image analysis problem. This approach rebuilds the way image processing is done from scratch, but at the same time it also based on concepts developed in computer vision. The deep learning approaches doesn't require a specific model describing the images it is meant to analyze, but rather make conclusions based on the previous images it has been exposed to.

The deep learning appraoch is good handling rare events in the data and when it trained correctly it is also capable of generalizing to detect new features. This may sound like magic, but this is also a well founded and structured approach to analyzing images. Care most however be taken not to over fit or to genralize to much. The models are never better than the data they have been exposed to.

Examples where deep learing is frequently used are to detect annomalies in the data or to label images based on their contents.

5.3.1 Characteristics

- · Results-driven
- · Biology 'inspired'
- Build both image processing and analysis from scratch

5.3.2 Examples

- · Captioning images
- · Identifying unusual events

5.4 Summary analysis approaches

These three approaches have their own advantages and disadvantages, therefore it is good to know them all to be able to select the adquate method for the task you have to solve. It is not unusual that you will have to use a mix of the approaches. It is important to be open minded and think outside the box. In the end, what matters is that you can provide a reliable analysis of your data.

CHAPTER

SIX

ON SCIENCE

6.1 What is the purpose?

• Discover and validate new knowledge

6.1.1 How?

- Use the scientific method as an approach to convince other people
- Build on the results of others so we don't start from the beginning

6.1.2 Important Points

- While qualitative assessment is important, it is difficult to reliably produce and scale
- Quantitative analysis is far from perfect, but provides metrics which can be compared and regenerated by anyone

Inspired by: imagej-pres

6.2 Science and Imaging

Images are great for qualitative analyses since our brains can quickly interpret them without large *programming* investements.

6.2.1 Proper processing and quantitative analysis is however much more difficult with images.

- If you measure a temperature, quantitative analysis is easy, 50K.
- If you measure an image it is much more difficult and much more prone to mistakes, subtle setup variations, and confusing analyses

6.2.2 Furthermore in image processing there is a plethora of tools available

- · Thousands of algorithms available
- · Thousands of tools
- Many images require multi-step processing
- · Experimenting is time-consuming

6.3 Important questions



Fig. 6.1: A realistic rendering of an ancient gold pendant.

- It is important to define how you want to use your images
- Nice rederings is mostly not sufficient for a publication

6.3.1 Initial questions

You need to ask yourself a number of questions before you start to plan an experiment that involves images. It is good to a at least have a plan about how you are going to analyze your images ones you have them. It is not always obvious how you get from the experiment idea, to performing the experiment and finally how you perform the analysis to obtain the information you are really interested in for your research project.

- What am I looking for?
- Is my original question specific or too general?
- How can I plan my experiment to make the analysis easier?
- How can I transfer the original question into the vocabulary of image analysis?
- How much a priori information about the sample do I have? Can it be used for the analysis?

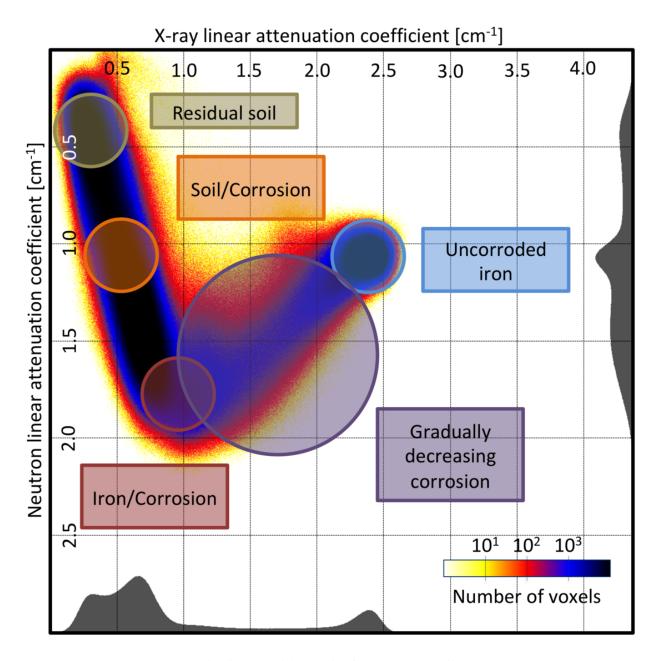
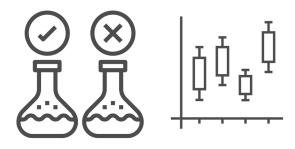
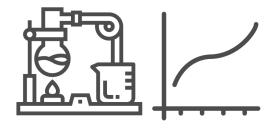


Fig. 6.2: Material analysis of a rusty roman nail.





Compare treatments

Understand and model processes



Inspect components

Fig. 6.3: Things a scientist wants to know from an experiment.

6.3.2 What is the purpose of the experiment

- 3D visualization
- Sample characterization
- Model process parameters
- etc.

6.3.3 What can we measure?

There is a leap between what you really want to know and what we actually can measure in the images.

Quantification from gray values	Quantification from shapes		
Material composition	Identify and count items		
Material transport	Measure volume		
	Characterize shape		

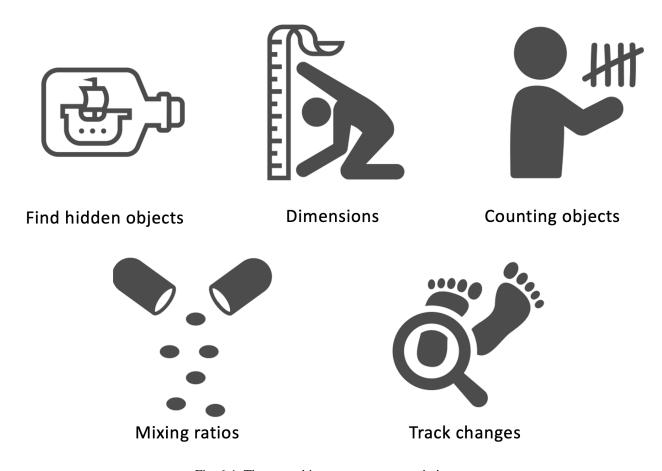


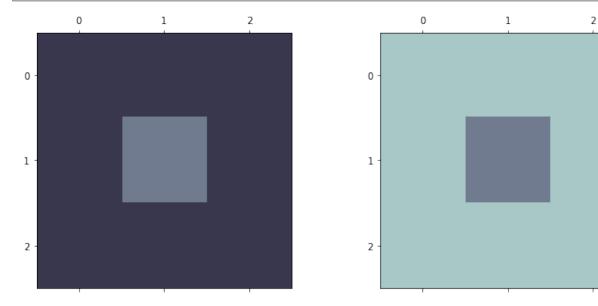
Fig. 6.4: These are things we can measure in images.

6.4 Why quantitative?

6.4.1 Human eyes have issues

Which center square seems brighter?

```
%matplotlib inline
import matplotlib.pyplot as plt
import numpy as np
xlin = np.linspace(-1,1, 3)
xx, yy = np.meshgrid(xlin, xlin)
img_a = 25*np.ones((3,3))
img_b = np.ones((3,3))*75
img_a[1,1] = 50
img_b[1,1] = 50
fig, (ax1, ax2) = plt.subplots(1,2, figsize = (12, 5));
ax1.matshow(img_a, vmin = 0, vmax = 100, cmap = 'bone');
ax2.matshow(img_b, vmin = 0, vmax = 100, cmap = 'bone');
```

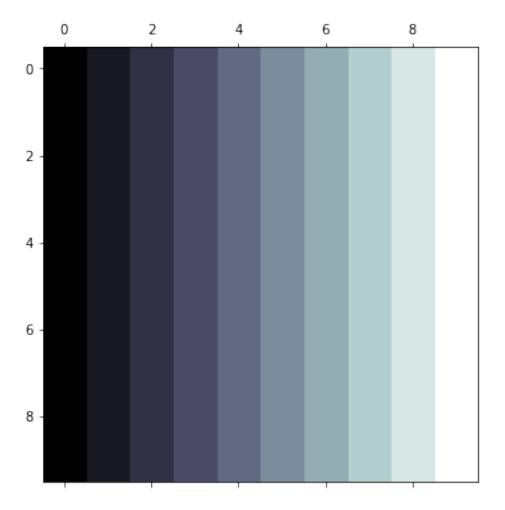


6.5 Intensity gradients

Are the intensities constant in the image?

```
%matplotlib inline
import matplotlib.pyplot as plt
import numpy as np
xlin = np.linspace(-1,1, 10)
xx, yy = np.meshgrid(xlin, xlin)

fig, ax1 = plt.subplots(1,1, figsize = (6, 6))
ax1.matshow(xx, vmin = -1, vmax = 1, cmap = 'bone');
```



6.6 Selecting the right tool for your task

6.6.1 Criteria

There are many questions you have to ask about the data and analysis task you are facing. Here are some relevant questions that will guide you towards the choice of tool(s) you need to use when you analyses the data.

- How many samples?
- How complex is the sample?
- Is human interpretation needed?
- What is the end product?
- Are there methods tools available?
- Will there be more similar data sets?

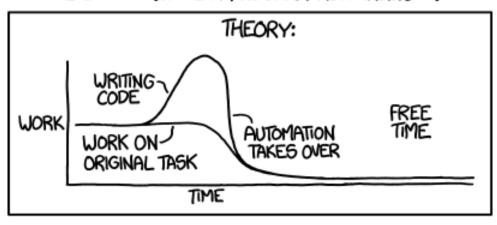
6.6.2 The choice

You have different options to choose the tool for your analysis.

- · Interactive tools
- Scripting using existing toolboxes
- Development of new algorithms Which one you actually select for your analysis workflow depends on many factors like the criteria listed in the previous section, but also on your experience, preferences in the group you are working with and so on.

In general, there is **no golden recipe**! You have to be flexible from task to task.

"I SPEND A LOT OF TIME ON THIS TASK. I SHOULD WRITE A PROGRAM AUTOMATING IT!"



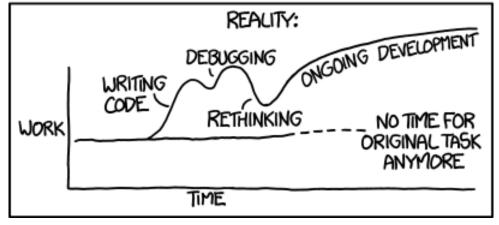


Fig. 6.5: It sometimes feels decievingly simple to implement a script that automates your task... be careful!

6.6.3 Criteria

- How many samples?
- How complex is the sample?
- Is human interpretation needed?
- What is the end product?
- Are there methods tools available?
- Will there be more similar data sets?

6.6.4 The choice

There is no golden recipe!

- · Interactive tools
- Scripting using existing toolboxes
- Development of new algorithms

6.7 Reproducibility vs. Repeatability

6.7.1 Reproducibility

To reproduce an experiment means that someone else can repeat your entire workflow from sample preparation and experiment to the analysis to obtain the same results you got in your report. This should be possible completely independently for an experiment to be called reproducible.

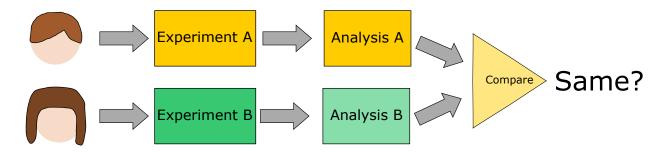


Fig. 6.6: A workflow describing the concept of reproducibility.

6.7.2 Repeatability

Repeatability is a more relaxed concept. It requires that someone else (or even yourself) can obtain the same results multiple time using the same data and analysis workflow. This means that there must very little room left for individual decisions that may have impact on the outcome.

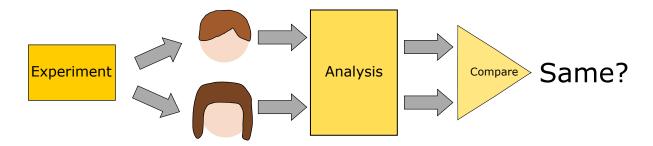


Fig. 6.7: A workflow describing the concept of reproducibility.

6.8 Reproducibility vs. Repeatability

Science demands repeatability! and really wants reproducability

- Experimental conditions can change rapidly and are difficult to make consistent
- Animal and human studies are prohibitively time consuming and expensive to reproduce
- Terabyte datasets cannot be easily passed around many different groups
- Privacy concerns can also limit sharing and access to data
- Science is already difficult enough
- Image processing makes it even more complicated
- Many image processing tasks are multistep, have many parameters, use a variety of tools, and consume a very long time

6.9 How can we keep track of everything for ourselves and others?

We can make the data analysis easy to repeat by an independent 3rd party

- Document the analysis steps
- Write clear and understandable code

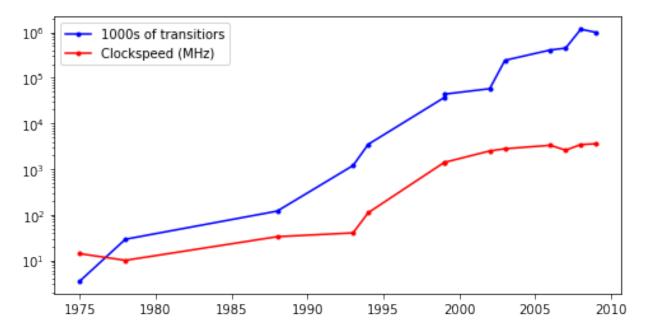
WORKFLOWS FOR IMAGE ANALYSIS

7.1 Computing has changed: Parallel

7.1.1 Moores Law

Transistors $\propto 2^{T/(18 \text{ months})}$

```
# Borrowed from https://gist.github.com/humberto-ortiz/de4b3a621602b78bf90d
moores_txt=["Id Name Year Count(1000s) Clock(MHz)\n",
                    MOS65XX 1975
                                            3.51
                                                            14\n",
       n 1
                   Intel8086 1978
                                           29.00
                                                           10\n",
       "2
                  MIPSR3000 1988
                                          120.00
                                                            33\n",
       "3
                    AMDAm486 1993
                                         1200.00
                                                            40\n",
                 NexGenNx586 1994
                                         3500.00
                                                          111\n",
       "5
                  AMDAthlon 1999
                                        37000.00
                                                         1400\n",
       "6 IntelPentiumIII 1999
                                       44000.00
                                                        1400\n",
                 PowerPC970 2002
                                        58000.00
       "7
                                                         2500\n",
       "8
                AMDAthlon64 2003
                                                        2800\n",
                                      243000.00
       "9
            IntelCore2Duo 2006
                                    410000.00
                                                       3330\n",
       "10
                   AMDPhenom 2007
                                       450000.00
                                                         2600\n",
       "11
                IntelCorei7 2008
                                    1170000.00
                                                         3460\n",
                IntelCorei5 2009
                                      995000.00
                                                         3600"1
sio_table = StringIO(''.join(moores_txt)); moore_df = pd.read_table(sio_table, sep =
\rightarrow'\s+', index_col = 0);
fig, ax1 = plt.subplots(1,1, figsize = (8, 4)); ax1.semilogy(moore_df['Year'], moore_
→df['Count(1000s)'], 'b.-', label = '1000s of transitiors'); ax1.semilogy(moore_df[
→'Year'], moore_df['Clock(MHz)'], 'r.-', label = 'Clockspeed (MHz)') ;ax1.legend(loc_
\rightarrow= 2);
```



Based on data from https://gist.github.com/humberto-ortiz/de4b3a621602b78bf90d

7.1.2 Why doesn't the clock rate follow Moore's law?

There are now many more transistors inside a single computer but the CPU clock rate hasn't increased. It is actually stagnating since the early 2000s. The reason for this is that the number of processing cores has started to increase. Each core is a CPU is an autonomous CPU which makes it possible to perform many individual tasks in parallel.

- Multiple Core
- Multiple CPUs
- · New modalities
 - GPUs provide many cores which operate at slow speed

The conclusion is that we need to make the processing on multiple Cores/CPUs possible. This can be done by chosing multithreaded modules for the processing. In the extreme case we need to implement new multi-process code for the analysis. How this is done depends in the target hardware. You will use different approaches for a CPU than you use in a cluster or in cloud computing. In general, we can conclude:

Parallel Code is important

7.2 Computing has changed: Cloud

Cloud computing is a type of service that has evolved from the big data era. Large companies have built up their analysis resources to match the need at peak demand. These resources were often underused which resulted in a new business model. Providing computing resources to external customers. In this way the customers only pay for the computing time they need and let someone else handle investments and system maintenance.

- Computer, servers, workstations are *wildly underused* (majority are <50%)
- Buying a big computer that sits idle most of the time is a waste of money
 - http://www-inst.eecs.berkeley.edu/~cs61c/sp14/



Fig. 7.1: Different cloud services where it is possible to buy CPU time.

- "The Case for Energy-Proportional Computing," Luiz André Barroso, Urs Hölzle, IEEE Computer, December 2007
- Traditionally the most important performance criteria was time, how fast can it be done
- With Platform as a service servers can be rented instead of bought
- Speed is still important but using cloud computing \$ / Sample is the real metric
- In Switzerland a PhD student is 400x as expensive per hour as an Amazon EC2 Machine
- · Many competitors keep prices low and offer flexibility

7.3 Workflow analysis

It sound like a great idea to perform tasks in parallel, but how do we bring the compoute to do so. The following example from real life is used to demonstrate how a greater task can be broken down into smaller piecese.

7.3.1 Simple Soup

We want to cook a simple soup. There is already a recipe telling us what to do to produce a soup. It is easy to follow the list, anyone with the right steps can execute and repeat (if not reproduce) the soup

- 1. Buy {carrots, peas, tomatoes} at market
- 2. then Buy meat at butcher
- 3. then Chop carrots into pieces
- 4. then Chop potatos into pieces
- 5. then Heat water
- 6. then Wait until boiling then add chopped vegetables
- 7. then Wait 5 minutes and add meat

All steps are done in a sequence to produce this soup. This would correspond to performing the task on a single CPU. Now, how can we change this?

7.3.2 More complicated soup

The following recipe is harder to follow and you need to carefully keep track of what is being performed. If you look at it in detail, you will see that some of the steps can be performed independently of the others. This is our opportunity to dispatch parallel tasks that would be running on different CPUs. In the kitchen, you could delegate tasks to other people and thus shorten the time until the soup is ready.

Steps 1-4 4. then Mix carrots with potatos $\rightarrow mix_1$ 4. then add egg to mix_1 and fry for 20 minutes 4. then Tenderize meat for 20 minutes 4. then add tomatoes to meat and cook for 10 minutes $\rightarrow mix_2$ 5. then Wait until boiling then add mix_1 6. then Wait 5 minutes and add mix_2

7.4 Using flow charts / workflows

The processing task can be visualize using flow charts to better get an overview of the steps to be done. A sequence has a trivial shape, but it hard to get the big picture as soon as you start creating more complicated schemes.

7.4.1 Workflow of the Simple Soup

In this example we use the graph plotting module graphviz to draw the flow chart of the soup example. Graphviz is only a plotting tool with no direct processing capacity.

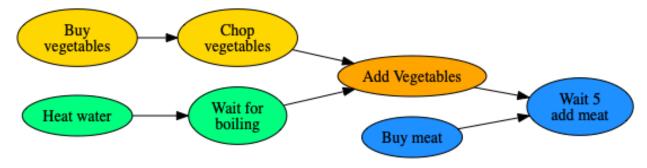


7.4.2 Workflows - the complicated soup

Clearly a linear set of instructions is ill-suited for even a fairly easy soup, it is then even more difficult when there are dozens of steps and different pathsways.

Furthermore a clean workflow allows you to better parallelize the task since it is clear which tasks can be performed independently.

(continued from previous page)



7.5 Directed Acyclical Graphs (DAG)

- We can represent almost any computation without loops as DAG.
- This allows us to break down a computation into pieces which can be carried out independently.

There are a number of tools which let us handle this issue.

- PyData Dask https://dask.pydata.org/en/latest/
- Apache Spark https://spark.apache.org/
- Spotify Luigi https://github.com/spotify/luigi
- Airflow https://airflow.apache.org/
- KNIME https://www.knime.com/
- Google Tensorflow https://www.tensorflow.org/
- Pytorch / Torch http://pytorch.org/

7.5.1 Concrete example - Creating a DAG

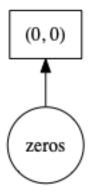
What is a DAG good for?

Create two variables

Let create a DAG to demonstrate how it works. This first piece creates two small images (5x5 pixels) each containing either '0's or '1's. We still haven't started any calculations using this DAG.

```
import dask.array as da

from dask.dot import dot_graph
image_1 = da.zeros((5,5), chunks = (5,5))
image_2 = da.ones((5,5), chunks = (5,5))
dot_graph(image_1.dask)
```

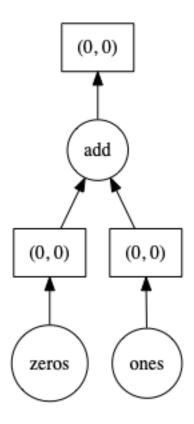


Add two variables

Adding two variables in a DAG looks like the code to add any variables. Now if we look at the graph created by this operation, we that there is a directed flow of the data in this graph.

$$Image_3 = Image_1 + Image_2 \\$$

```
image_3 = image_1 + image_2
dot_graph(image_3.dask)
```

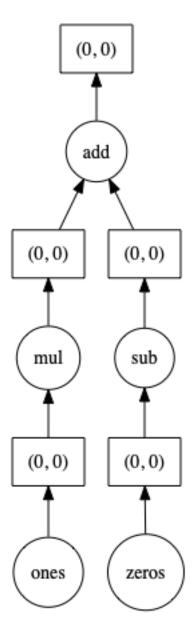


A more complicated operation

Now lets make a more complicated calculation like:

$$Image_4 = (image_1 - 10) + (image_2 * 50)$$

```
image_4 = (image_1-10) + (image_2*50)
dot_graph(image_4.dask)
```



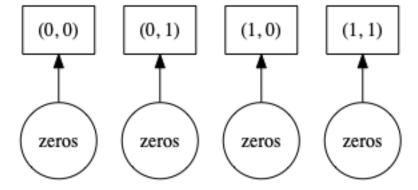
We can here see that there are two clear branches in the flow chart. This indicates that here is an opportunity to perform these tasks in parallel.

7.5.2 Let's go big

Now let's see where this can be really useful. Dask allows you to split the data into smaller pieces (chunks). In the example below we have created a 1024x1024 pixels image and also told dask that this image should be divided into chunks of 512x512 pixels.

Creating a large image with chunks

```
import dask.array as da
from dask.dot import dot_graph
image_1 = da.zeros((1024, 1024), chunks = (512, 512))
image_2 = da.ones((1024, 1024), chunks = (512, 512))
dot_graph(image_1.dask)
```



You see now that there are four graph created. The boxes on the top indicates the location of the chunk in the large image.

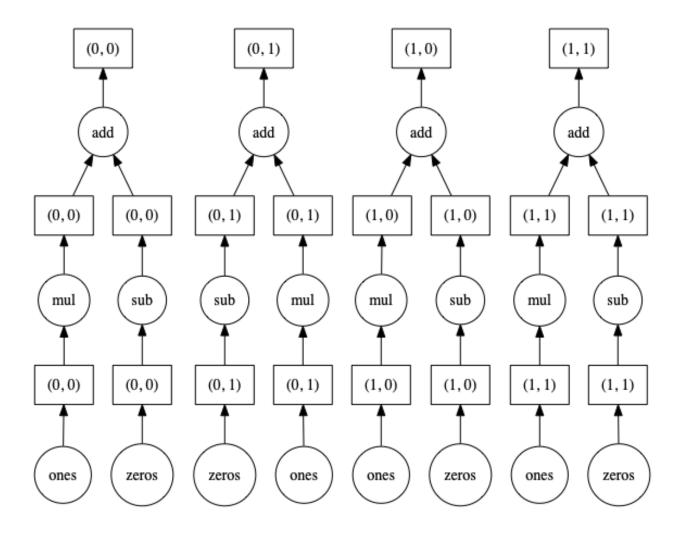
Computing something with a larger image

If we now apply the "complicated" calculation

$$Image_4 = (image_1 - 10) + (image_2 * 50)$$

on the large image with chunks, we see that the code hasn't changed at all. When we look at the graph generated with this data, we see that there are four graphs repeating the same operations. One graph for each chunk we defined in the data.

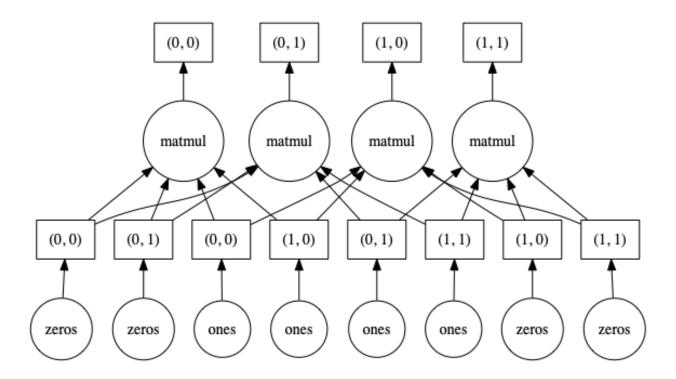
```
image_4 = (image_1-10) + (image_2*50)
dot_graph(image_4.dask)
```



7.5.3 Matrix multiplication in a DAG

A matrix multiplication is a more complicated operation than the previous exmaples. This operation needs information from all the other chunks to calulate the current chunk. This can also be seen in the wiring of the graph below.

```
image_5 = da.matmul(image_1, image_2)
dot_graph(image_5.dask)
```

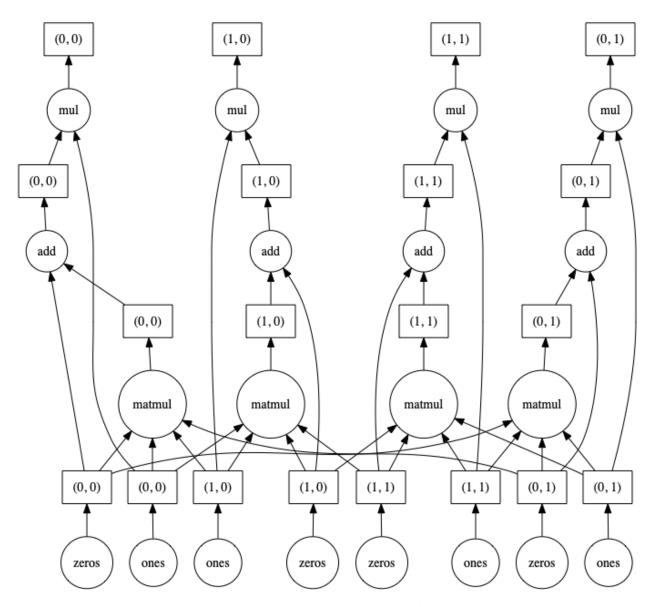


7.5.4 An even more complicated computation

Combining different operations into a final calculation like

$$Image_6 = (Image_1 \cdot Image_2 + Image_1) \circ Image_2$$

image_6 = (da.matmul(image_1, image_2)+image_1)*image_2
dot_graph(image_6.dask)



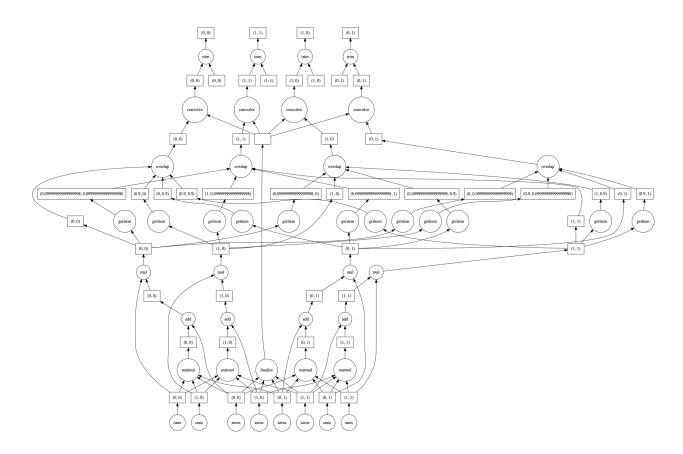
We see here that the convolution part connects all branches of the graph which mean all processing must be synchronized here. Afterwards there are no connections and each sub graph can work independently.

7.5.5 Convolution using a DAG

Convolution makes life harder for a DAG. There is a need for an overlap between the chunks to avoid boundary effects. These boundary effects are something we will look into in next week's lecture about filters.

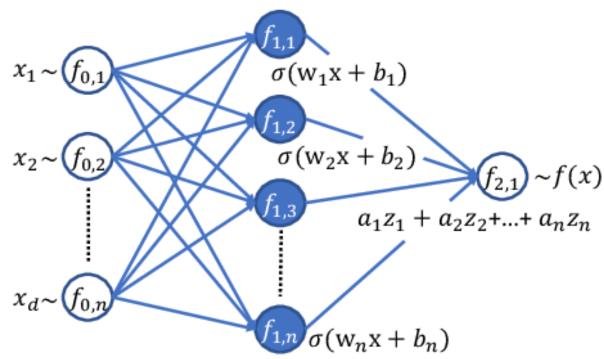
$$Image_7 = Image_6 * Image_1$$

```
import dask_ndfilters as da_ndfilt
image_7 = da_ndfilt.convolve(image_6, image_1)
dot_graph(image_7.dask)
```



7.6 Deep Learning

We won't talk too much about deep learning now, but it certainly shows why DAGs are so important.



7.6. Deep Learning 73

Quantitative Big Imaging - Introduction

The steps above are simple toys compared to what tools are already in use for machine learning https://keras.io/api/utils/model_plotting_utils/

CHAPTER

EIGHT

SUMMARY

In this lecture we saw that:

- Images revieal information about different samples
- Images are a signals that needs to be quantitatively analyzed
- Science with images is a non-trivial task
- Proper workflows are required for efficient analysis repeatable analysis.

8.1 Next weeks lecture

- Noise
- Filters
- Filter evaluation