
Quantitative Big Imaging - Basic segmentation

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Part 1: Image formation and thresholding

**CHAPTER
ONE**

TODAY'S LECTURE

- Motivation
- Qualitative Approaches
- Thresholding
- Other types of images
- Selecting a good threshold
- Implementation
- Morphology
- Contouring / Mask Creation

**CHAPTER
TWO**

APPLICATIONS

- Simple two-phase materials (bone, cells, etc)
 - Beyond 1 channel of depth
- Multiple phase materials
- Filling holes in materials
- Segmenting Fossils
- Attempting to segment the cortex in brain imaging

LITERATURE / USEFUL REFERENCES

- John C. Russ, “The Image Processing Handbook”,(Boca Raton, CRC Press)
- Available [online](#) within domain [ethz.ch](#) (or [proxy.ethz.ch](#) / public VPN)

3.1 Models / ROC Curves

- Julia Evans - Recalling with Precision
- Stripe’s Next Top Model

MOTIVATION: WHY DO WE DO IMAGING EXPERIMENTS?

4.1 Exploratory

- To visually, qualitatively examine samples and differences between them
- No prior knowledge or expectations

4.2 To test a hypothesis

Quantitative assessment coupled with statistical analysis

- Does temperature affect bubble size?
- Is this gene important for cell shape and thus mechanosensation in bone?
- Does higher canal volume make bones weaker?
- Does the granule shape affect battery life expectancy?

4.3 What we are looking at?

4.4 What we get from the imaging modality

```
%matplotlib inline
from skimage.io import imread
from skimage.color import rgb2gray
import matplotlib.pyplot as plt
dkimg = imread("figures/Average_prokaryote_cell.jpg")
plt.matshow(rgb2gray(dkimg), cmap = 'bone');
```

```
-----
ModuleNotFoundError                                     Traceback (most recent call last)
<ipython-input-1-e99542f45115> in <module>
      1 get_ipython().run_line_magic('matplotlib', 'inline')
----> 2 from skimage.io import imread
      3 from skimage.color import rgb2gray
      4 import matplotlib.pyplot as plt
      5 dkimg = imread("figures/Average_prokaryote_cell.jpg")

ModuleNotFoundError: No module named 'skimage'
```

4.5 To test a hypothesis

We perform an experiment bone to see how big the cells are inside the tissue:



4.5.1 $2560 \times 2560 \times 2160 \times 32 \text{ bit} = 56\text{GB} / \text{sample}$



4.5.2 20h of computer time later ...

4.5.3 Way too much data, we need to reduce

4.6 What did we want in the first place?

4.6.1 *Single numbers:*

- volume fraction,
- cell count,
- average cell stretch,
- cell volume variability

4.7 Why do we perform segmentation?

- In model-based analysis every step we perform, simple or complicated is related to an underlying model of the system we are dealing with
- *Occam's Razor* is very important here : The simplest solution is usually the right one
- Bayesian, neural networks optimized using genetic algorithms with Fuzzy logic has a much larger parameter space to explore, establish sensitivity in, and must perform much better and be tested much more thoroughly than thresholding to be justified.
- We will cover some of these techniques in the next 2 lectures since they can be very powerful particularly with unknown data

CHAPTER
FIVE

REVIEW: FILTERING AND IMAGE ENHANCEMENT (LAST WEEK)

This was a noise process which was added to otherwise clean imaging data

$$I_{measured}(x, y) = I_{sample}(x, y) + \text{Noise}(x, y)$$

- What would the perfect filter be

$$\text{Filter} * I_{sample}(x, y) = I_{sample}(x, y)$$

$$\text{Filter} * \text{Noise}(x, y) = 0$$

$$\text{Filter} * I_{measured}(x, y) = \text{Filter} * I_{real}(x, y) + \text{Filter} * \text{Noise}(x, y) \rightarrow \mathbf{I}_{sample}(\mathbf{x}, \mathbf{y})$$

What **most filters** end up doing $\$ \text{Filter} * I_{measured}(x, y) = 90\% I_{real}(x, y) + 10\% \text{Noise}(x, y) \$$

What **bad filters** do $\$ \text{Filter} * I_{measured}(x, y) = 10\% I_{real}(x, y) + 90\% \text{Noise}(x, y) \$$

QUALITATIVE METRICS: WHAT DID PEOPLE USE TO DO?

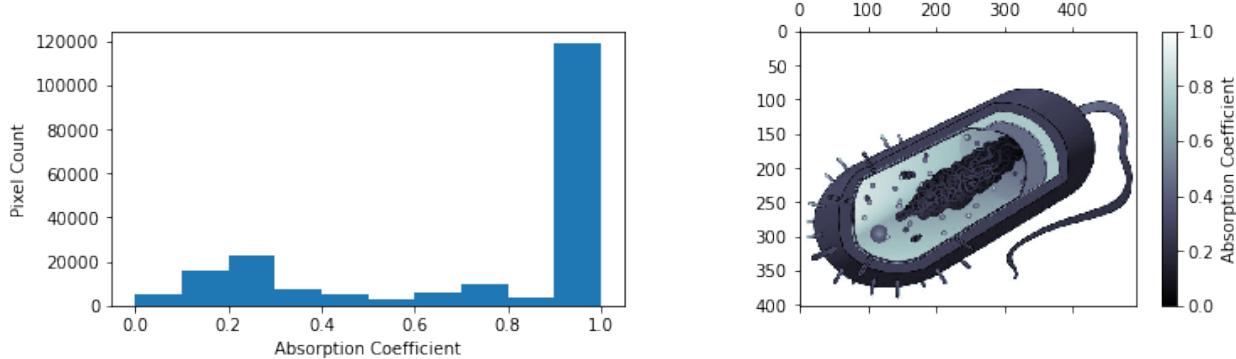
- What comes out of our detector / enhancement process

```
%matplotlib inline
from skimage.io import imread
from skimage.color import rgb2gray
import matplotlib.pyplot as plt
```

```
dkimg = rgb2gray(imread("figures/Average_prokaryote_cell.jpg"))
fig, (ax_hist, ax_img) = plt.subplots(1, 2, figsize = (12,3))

ax_hist.hist(dkimg.ravel())
ax_hist.set_xlabel('Absorption Coefficient')
ax_hist.set_ylabel('Pixel Count')

m_show_obj = ax_img.matshow(dkimg, cmap = 'bone')
cb_obj = plt.colorbar(m_show_obj)
cb_obj.set_label('Absorption Coefficient')
```



6.1 Identify objects by eye

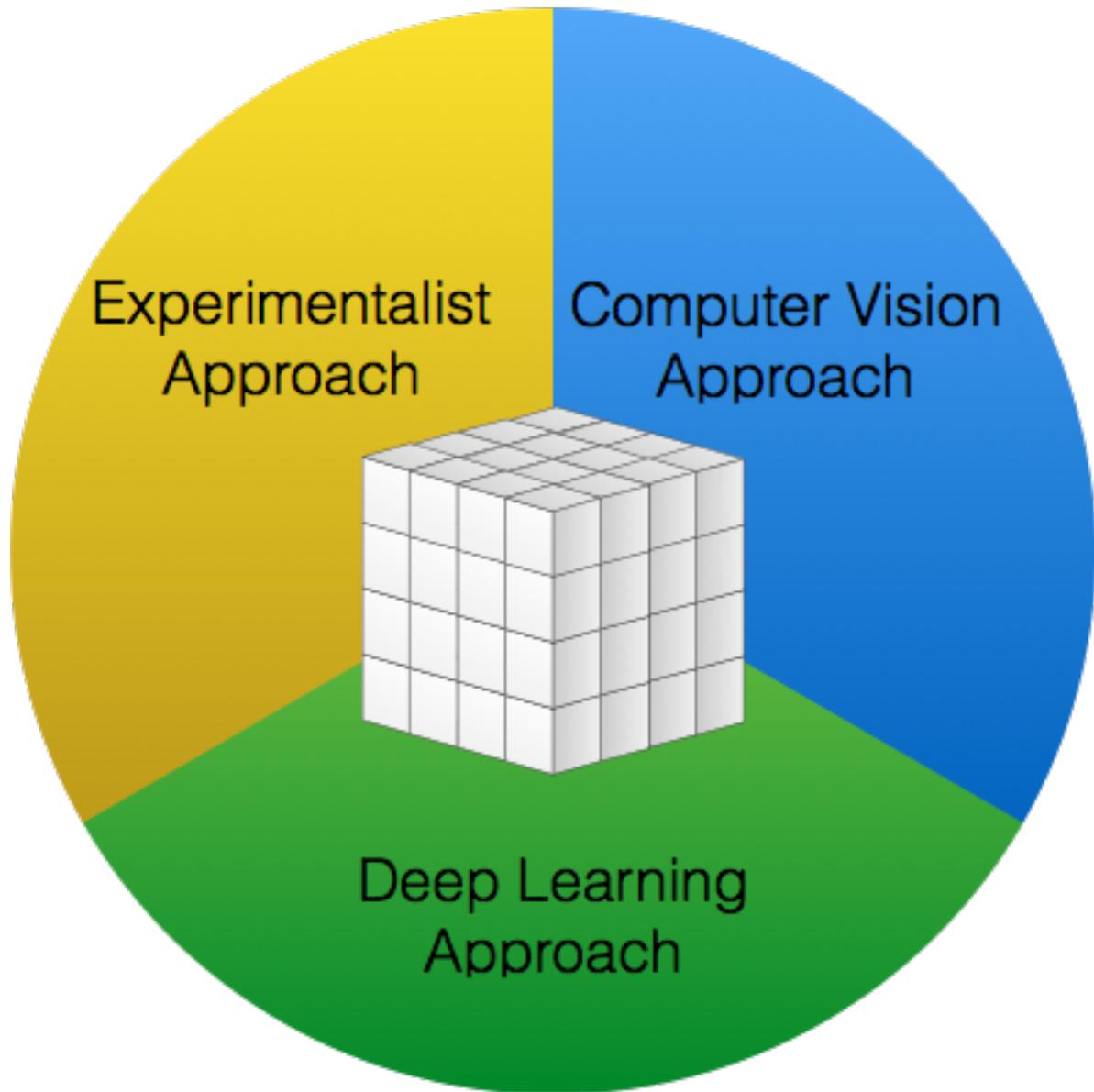
- Count, describe qualitatively: “many little cilia on surface”, “long curly flagellum”, “elongated nuclear structure”

6.2 Morphometrics

- Trace the outline of the object (or sub-structures)
- Can calculate the area by using equal-weight-paper
- Employing the “cut-and-weigh” method

SEGMENTATION APPROACHES

They match up well to the world view / perspective



7.1 Segmentation approaches

Problem-driven

- Top-down
- *Reality* Model-based

MODEL-BASED ANALYSIS

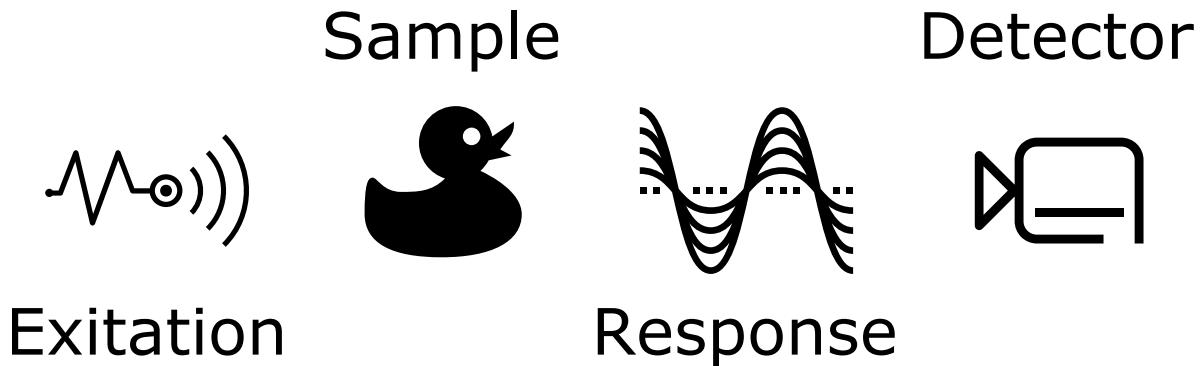


Fig. 8.1: The elements of the image formation process.

- Many different imaging modalities (μ CT to MRI to Confocal to Light-field to AFM).
- Similarities in underlying equations
- different coefficients, units, and mechanism

$$I_{measured}(\vec{x}) = F_{system}(I_{stimulus}(\vec{x}), S_{sample}(\vec{x}))$$

8.1 Direct Imaging (simple)

In many setups there is un-even illumination caused by incorrectly adjusted equipment and fluctuations in power and setups

$$F_{system}(a, b) = a * b$$

$$I_{stimulus} = \text{Beam}_{profile} \quad S_{system} = \alpha(\vec{x}) \longrightarrow \alpha(\vec{x}) = \frac{I_{measured}(\vec{x})}{\text{Beam}_{profile}(\vec{x})}$$

```
%matplotlib inline
from skimage.io import imread
from skimage.color import rgb2gray
import matplotlib.pyplot as plt
from skimage.morphology import disk
from scipy.ndimage import zoom
import numpy as np
```

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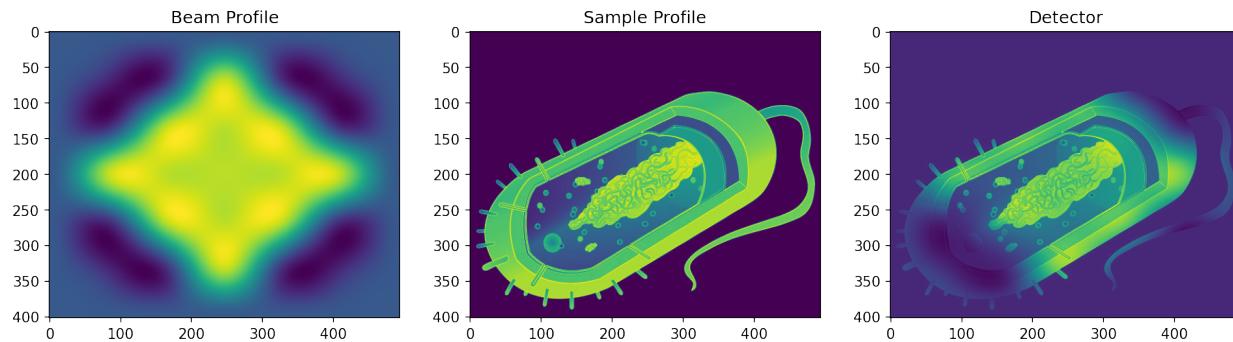
```
cell_img = 1-rgb2gray(imread("figures/Average_prokaryote_cell.jpg"))
s_beam_img = np.pad(disk(2)/1.0, [[1,1], [1,1]], mode = 'constant', constant_values = 0.2)
beam_img = zoom(s_beam_img, [cell_img.shape[0]/7.0, cell_img.shape[1]/7.0])

fig, (ax_beam, ax_img, ax_det) = plt.subplots(1, 3, figsize = (15,6), dpi=150)

ax_beam.imshow(beam_img, cmap = 'viridis'); ax_beam.set_title('Beam Profile')

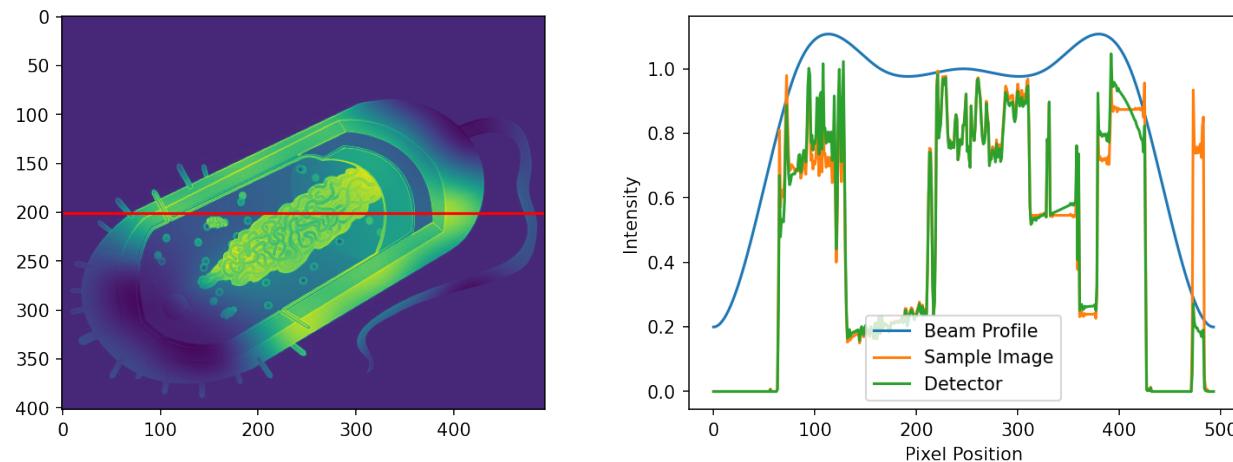
ax_img.imshow(cell_img, cmap = 'viridis'); ax_img.set_title('Sample Profile')

ax_det.imshow(cell_img*beam_img, cmap = 'viridis'); ax_det.set_title('Detector');
```



8.1.1 Profiles across the image

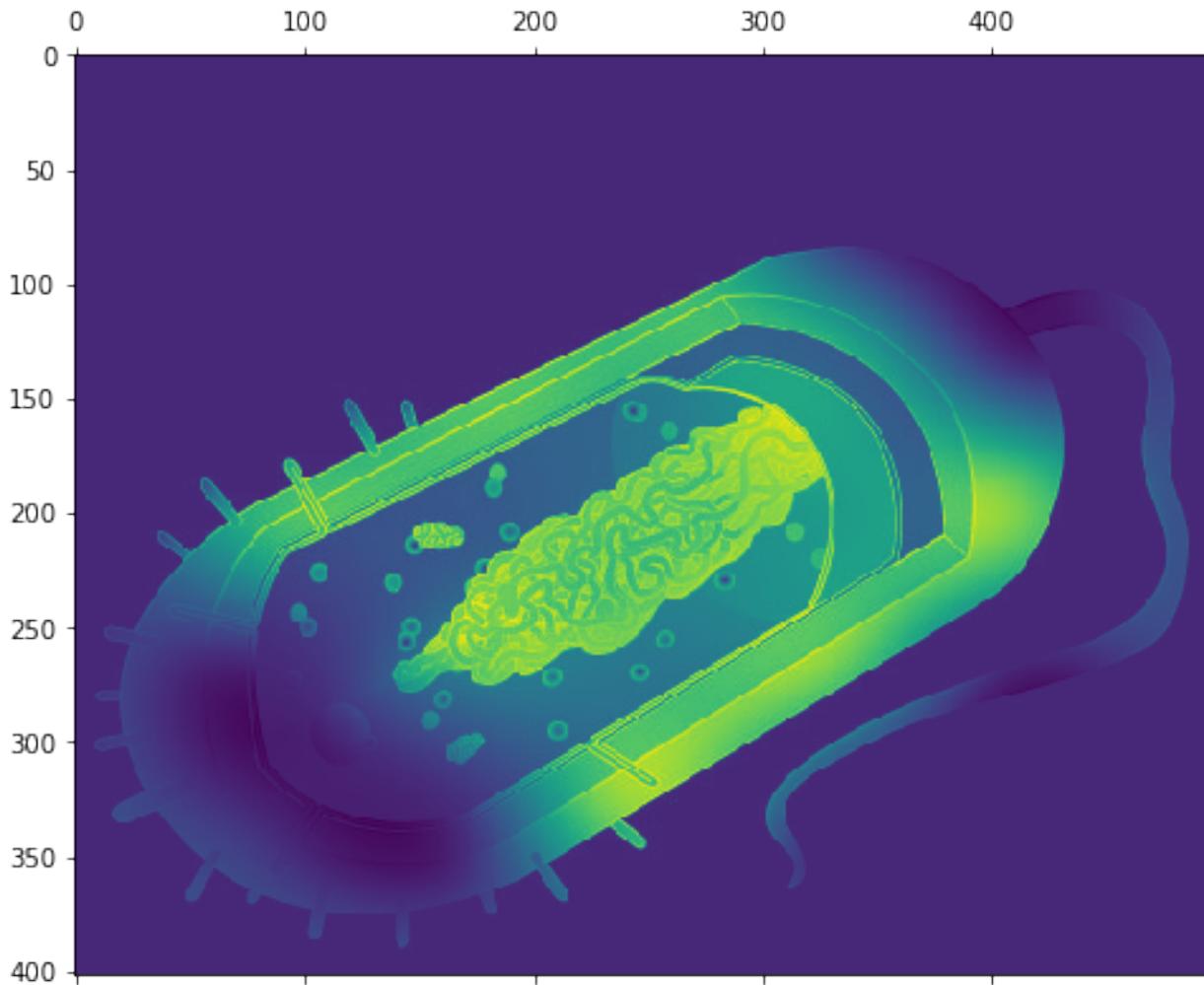
```
fig, ax = plt.subplots(1, 2, figsize = (12,4), dpi=150)
ax[0].imshow(cell_img*beam_img); ax[0].hlines(beam_img.shape[0]/2,xmin=0,xmax=beam_img.shape[1]-1,color='red')
ax[1].plot(beam_img[beam_img.shape[0]//2], label = 'Beam Profile')
ax[1].plot(cell_img[beam_img.shape[0]//2], label = 'Sample Image')
ax[1].plot((cell_img*beam_img)[beam_img.shape[0]//2], label = 'Detector')
ax[1].set_ylabel('Intensity'); ax[1].set_xlabel('Pixel Position'); ax[1].legend(loc="lower center");
```



8.2 Inhomogeneous illumination

- Frequently there is a fall-off of the beam away from the center (as is the case of a Gaussian beam which frequently shows up for laser systems).
- This can make extracting detail away from the center

```
fig, ax1 = plt.subplots(1,1, figsize = (8,8))
ax1.matshow(cell_img*beam_img,cmap = 'viridis');
```



8.3 Absorption Imaging (X-ray, Ultrasound, Optical)

8.3.1 For absorption/attenuation imaging → Beer-Lambert Law

$$I_{detector} = \underbrace{I_{source}}_{I_{stimulus}} \underbrace{e^{-\alpha d}}_{S_{sample}}$$

Different components have a different α based on the strength of the interaction between the light and the chemical /

Quantitative Big Imaging - Basic segmentation

nuclear structure of the material

$$I_{sample}(x, y) = I_{source} \cdot e^{-\alpha(x, y) \cdot d}$$

$$\alpha = f(N, Z, \sigma, \dots)$$

For segmentation this model is:

- there are 2 (or more) distinct components that make up the image
- these components are distinguishable by their values (or vectors, colors, tensors, ...)

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

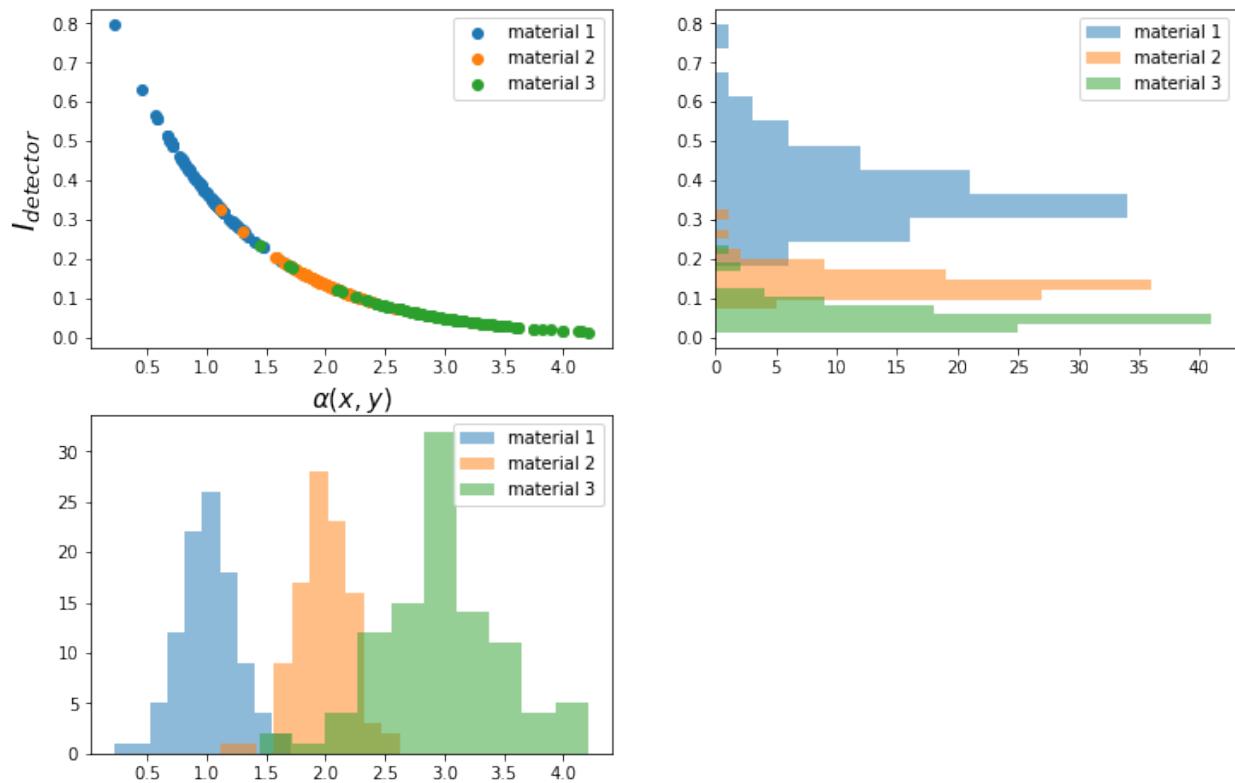
```
I_source = 1.0
d = 1.0
alpha_1 = np.random.normal(1, 0.25, size = 100)
alpha_2 = np.random.normal(2, 0.25, size = 100)
alpha_3 = np.random.normal(3, 0.5, size = 100)

abs_df = pd.DataFrame([dict(alpha = c_x, material = c_mat) for c_vec, c_mat in zip([alpha_1, alpha_2, alpha_3], ['material 1', 'material 2', 'material 3']) for c_x in c_vec])

abs_df['I_detector'] = I_source*np.exp(-abs_df['alpha']*d)
abs_df.sample(5)
```

	alpha	material	I_detector
227	2.899166	material 3	0.055069
60	1.408457	material 1	0.244520
197	1.577132	material 2	0.206567
28	1.189721	material 1	0.304306
167	1.501914	material 2	0.222704

```
fig, ((ax1, ax2), (ax3, ax4)) = plt.subplots(2, 2, figsize = (12, 8))
for c_mat, c_df in abs_df.groupby('material'):
    ax1.scatter(x = c_df['alpha'],
                y = c_df['I_detector'],
                label = c_mat)
    ax3.hist(c_df['alpha'], alpha = 0.5, label = c_mat)
    ax2.hist(c_df['I_detector'], alpha = 0.5, label = c_mat, orientation="horizontal")
    ax1.set_xlabel('$\\alpha(x,y)$', fontsize = 15); ax1.set_ylabel('$I_{detector}$', fontsize = 18)
    ax1.legend(); ax2.legend(); ax3.legend(loc = 0); ax4.axis('off');
```



EXAMPLE MAMMOGRAPHY

Mammographic imaging is an area where model-based absorption imaging is problematic. Even if we assume a constant illumination (*rarely* the case),

$$\begin{aligned} I_{detector} &= \frac{\underbrace{I_{source}}_{I_{stimulus}}}{\underbrace{S_{sample}}} \exp(-\alpha d) \\ &\downarrow \\ I_{detector} &= \exp(-\alpha(x, y)d(x, y)) \\ &\downarrow \\ I_{detector} &= \exp\left(-\int_0^l \alpha(x, y, z)dz\right) \end{aligned}$$

9.1 Problems to interpret radiography images

Specifically the problem is related to the inability to separate the

- α - attenuation
- d - thickness terms.

We model a basic breast volume as a half sphere with a constant absorption factor: $\alpha(x, y, z) = 0.01$

→ The \int then turns into a Σ in discrete space

- Air
- Breast tissue

9.2 Building a breast phantom

```
%matplotlib inline
import matplotlib.pyplot as plt
import numpy as np
from skimage.morphology import ball

# For the 3D rendering
import plotly.offline as py
from plotly.figure_factory import create_trisurf
from skimage.measure import marching_cubes
```

```

breast_mask = ball(50)[:,50:] # This is our model

# just for 3D rendering, don't worry about it
py.init_notebook_mode()
vertices, simplices, _, _ = marching_cubes(breast_mask>0)
x,y,z = zip(*vertices)
fig = create_trisurf(x=x, y=y, z=z,
                      plot_edges=False,
                      simplices=simplices,
                      title="Breast Phantom")
py.iplot(fig)

```

9.2.1 Transmission image of the breast phantom

```

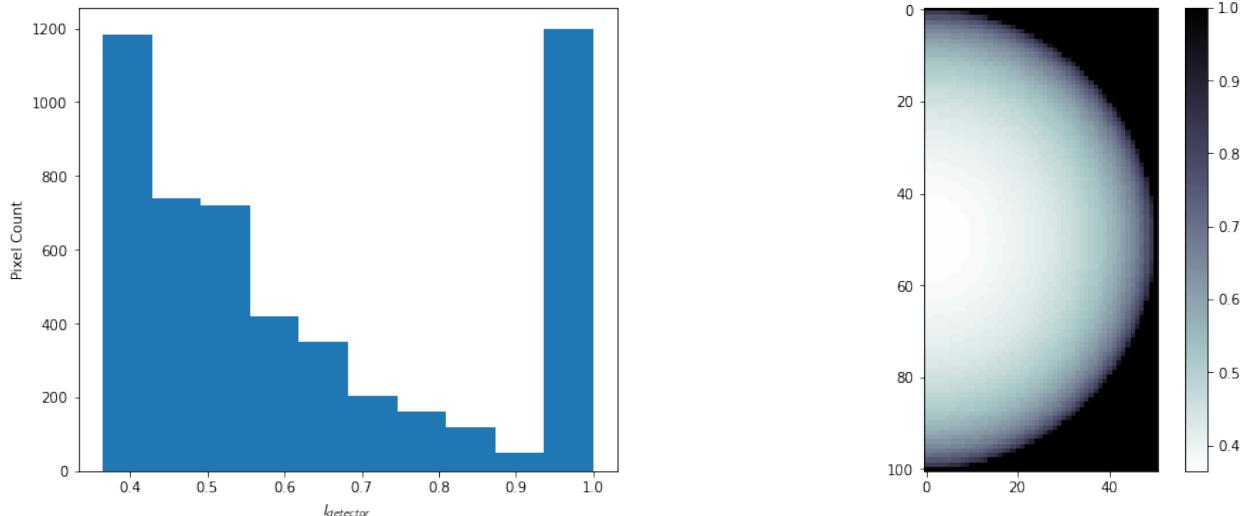
breast_alpha = 1e-2                                # The attenuation coefficient
breast_vol   = breast_alpha*breast_mask            # Scale the image intensity by
# attenuation coefficient
i_detector   = np.exp(-np.sum(breast_vol,2))      # Compute the transmission through the
# phantom

fig, (ax_hist, ax_breast) = plt.subplots(1, 2, figsize = (15,6))

b_img_obj = ax_breast.imshow(i_detector, cmap = 'bone_r'); plt.colorbar(b_img_obj)

ax_hist.hist(i_detector.flatten()); ax_hist.set_xlabel('$I_{detector}$'); ax_hist.set_
#ylabel('Pixel Count');

```



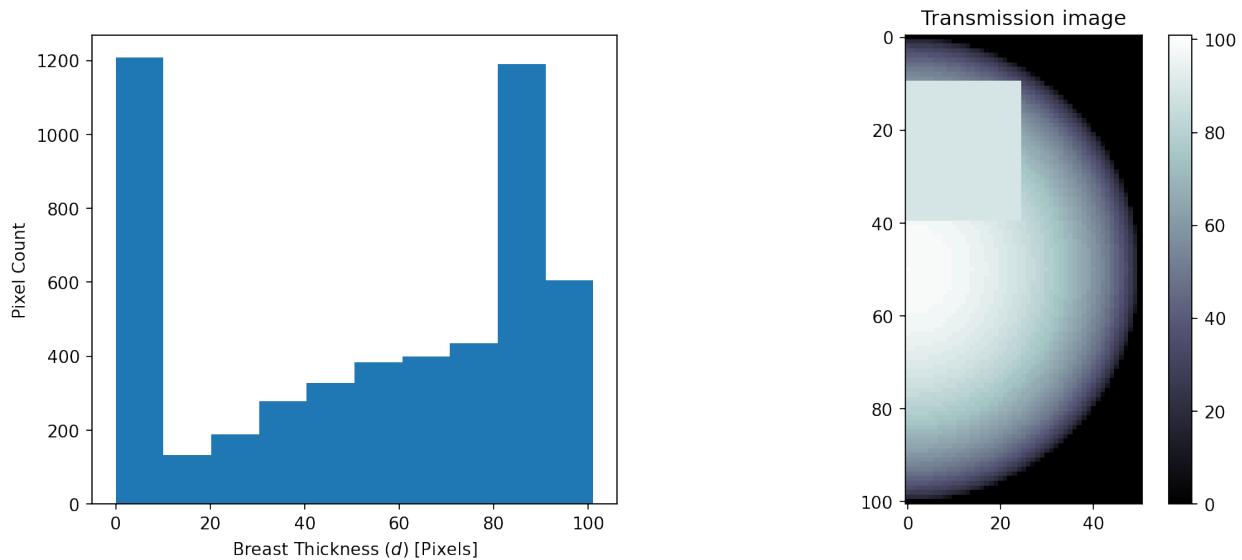
9.2.2 Compute the thickness

If we know that α is constant we can reconstruct the thickness d from the image: $d = -\log(I_{detector})$

```
breast_thickness = -np.log(i_detector)/breast_alpha
fig, (ax_hist, ax_breast) = plt.subplots(1, 2, figsize = (12,5), dpi=150)

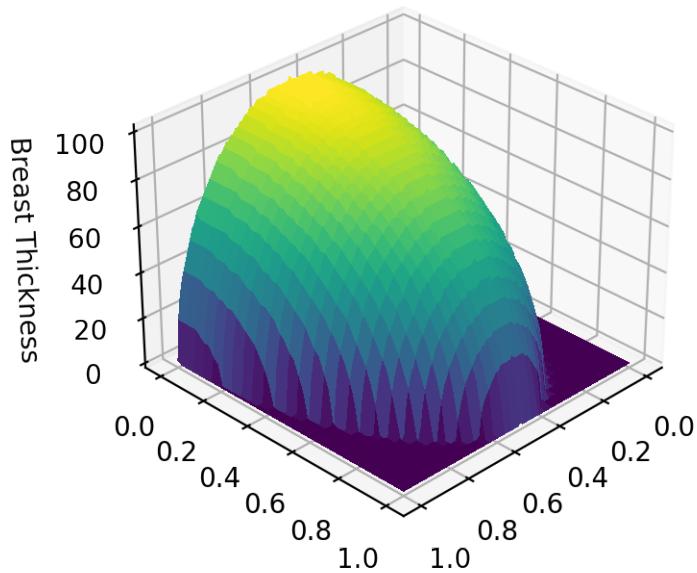
b_img_obj = ax_breast.imshow(breast_thickness, cmap = 'bone'); ax_breast.set_title(
    'Transmission image')
plt.colorbar(b_img_obj)

ax_hist.hist(breast_thickness.flatten()); ax_hist.set_xlabel('Breast Thickness ($d$) [Pixels]')
ax_hist.set_ylabel('Pixel Count');
```



9.2.3 Visualizing the thickness

```
from mpl_toolkits.mplot3d import Axes3D
fig = plt.figure(figsize = (8, 4), dpi = 200)
ax = fig.gca(projection='3d')
# Plot the surface.
yy, xx = np.meshgrid(np.linspace(0, 1, breast_thickness.shape[1]),
                      np.linspace(0, 1, breast_thickness.shape[0]))
surf = ax.plot_surface(xx, yy, breast_thickness, cmap=plt.cm.viridis,
                       linewidth=0, antialiased=False)
ax.view_init(elev = 30, azim = 45)
ax.set_zlabel('Breast Thickness');
```



9.3 What if α is not constant?

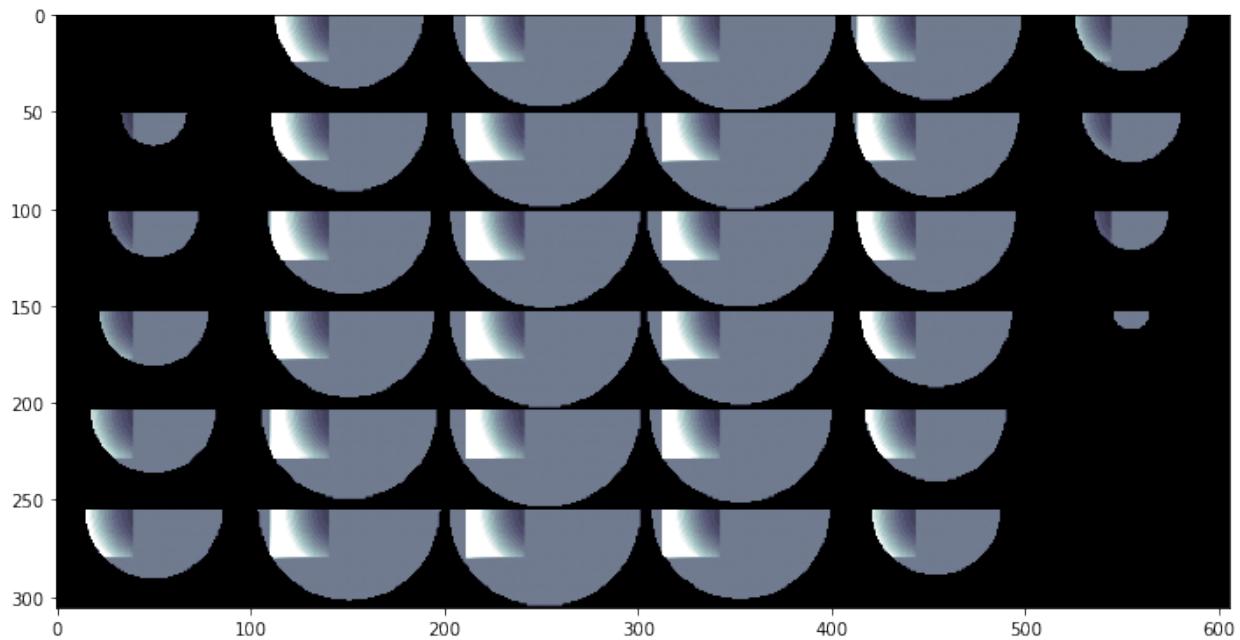
We run into problems when the α is no longer constant.

- For example if we place a dark lump in the center of the breast.
- It is **impossible** to tell if the breast is *thicker* or if the lump inside is *denser*.

For the lump below we can see on the individual slices of the sample that the lesion appears quite clearly and is very strangely shaped.

```
breast_vol = breast_alpha*breast_mask
renorm_slice = np.sum(breast_mask[10:40, 0:25], 2)/np.sum(breast_mask[30, 10])
breast_vol[10:40, 0:25] /= np.stack([renorm_slice]*breast_vol.shape[2], -1)

from skimage.util import montage as montage2d
fig, ax1 = plt.subplots(1,1, figsize = (12, 12))
ax1.imshow(montage2d(breast_vol.swapaxes(0,2).swapaxes(1,2)[::3]).transpose(),
           cmap = 'bone', vmin = breast_alpha*.8, vmax = breast_alpha*1.2);
```



9.3.1 Looking at the thickness again

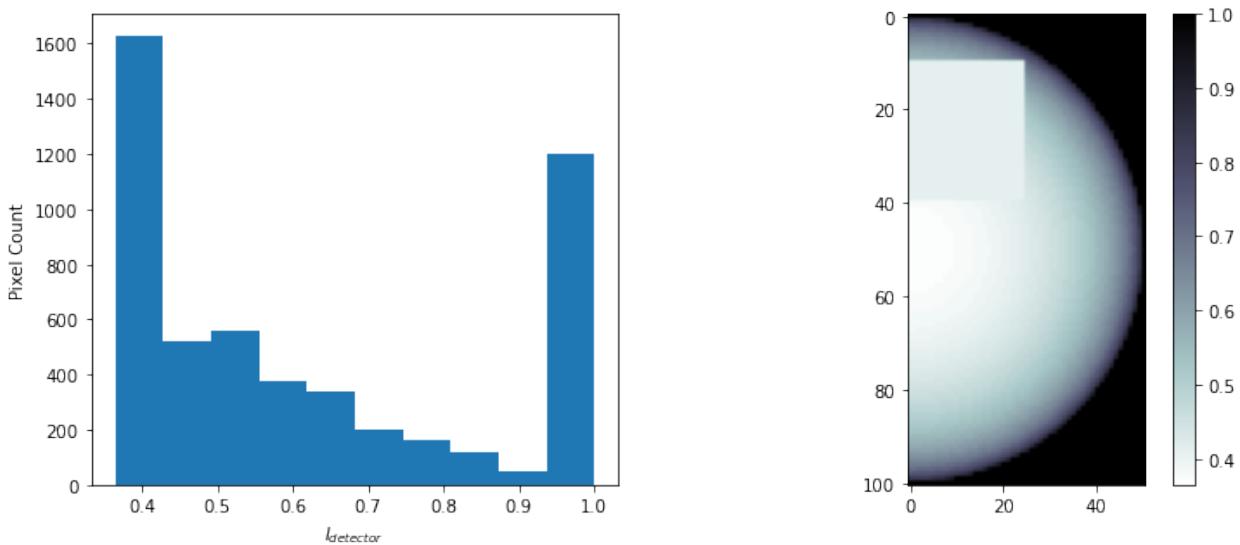
When we make the projection and apply Beer's Law we see that it appears as a relatively constant region in the image

```
i_detector = np.exp(-np.sum(breast_vol, 2))

fig, (ax_hist, ax_breast) = plt.subplots(1, 2, figsize = (12,5), dpi=150)

b_img_obj = ax_breast.imshow(i_detector, cmap = 'bone_r')
plt.colorbar(b_img_obj)

ax_hist.hist(i_detector.flatten())
ax_hist.set_xlabel('$I_{detector}$')
ax_hist.set_ylabel('Pixel Count');
```



9.3.2 An anomaly in the thickness reconstruction

It appears as a flat constant region in the thickness reconstruction.

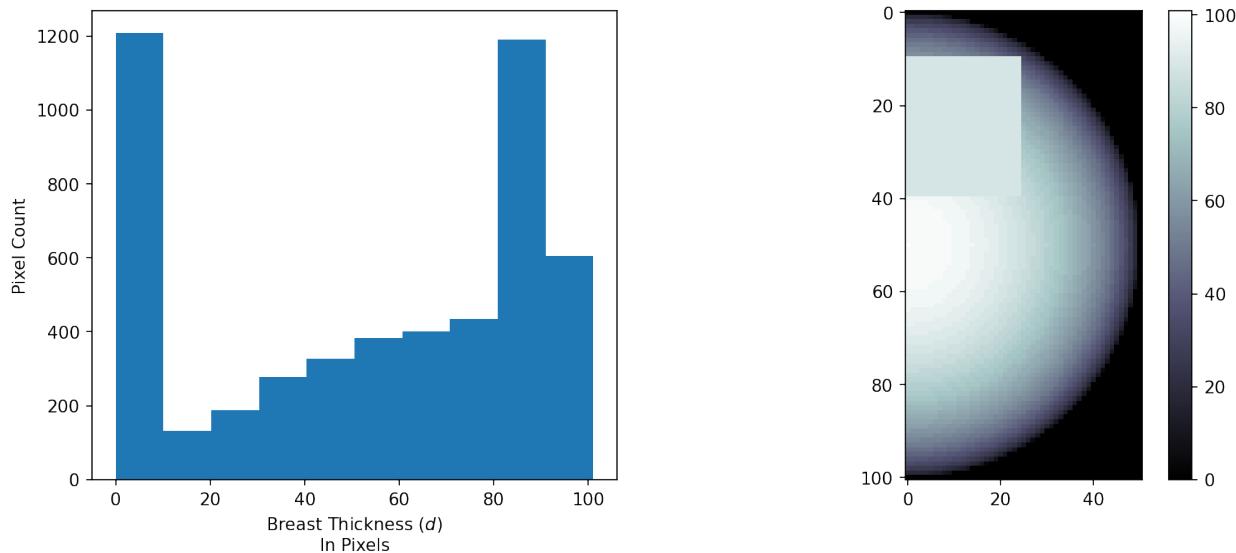
So we fundamentally from this single image cannot answer:

- is the breast oddly shaped?
- or does it have an possible tumor inside of it?

```
breast_thickness = -np.log(i_detector)/1e-2
fig, (ax_hist, ax_breast) = plt.subplots(1, 2, figsize = (12,5), dpi=150)

b_img_obj = ax_breast.imshow(breast_thickness, cmap = 'bone')
plt.colorbar(b_img_obj)

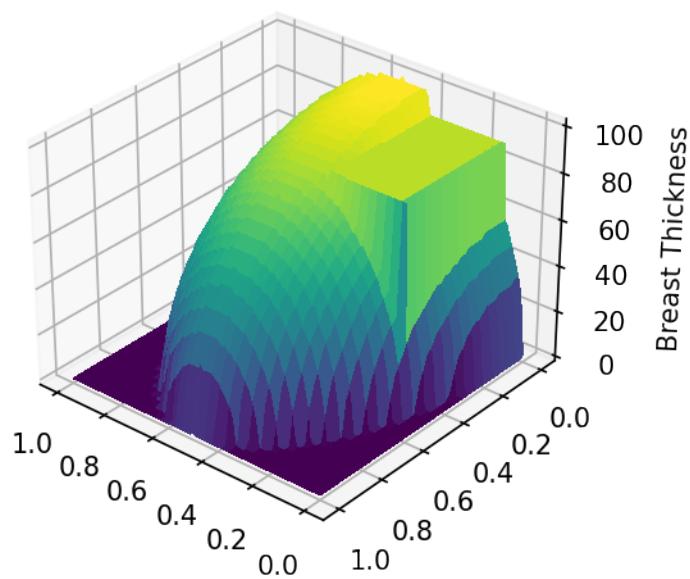
ax_hist.hist(breast_thickness.flatten())
ax_hist.set_xlabel('Breast Thickness ($d$)\nIn Pixels')
ax_hist.set_ylabel('Pixel Count');
```



9.3.3 Looking at the thickness profile again

```
from mpl_toolkits.mplot3d import Axes3D
fig = plt.figure(figsize = (8, 4), dpi = 150)
ax = fig.gca(projection='3d')

# Plot the surface.
yy, xx = np.meshgrid(np.linspace(0, 1, breast_thickness.shape[1]),
                     np.linspace(0, 1, breast_thickness.shape[0]))
surf = ax.plot_surface(xx, yy, breast_thickness, cmap=plt.cm.viridis,
                      linewidth=0, antialiased=False)
ax.view_init(elev = 30, azim = 130)
ax.set_zlabel('Breast Thickness');
```



SEGMENTATION

10.1 Where does segmentation get us?

We can convert a decimal value or something even more complicated like

- 3 values for RGB images,
- a spectrum for hyperspectral imaging,
- or a vector / tensor in a mechanical stress field

To a single or a few discrete values:

- usually true or false,
- but for images with phases it would be each phase, e.g. bone, air, cellular tissue.

2560 x 2560 x 2160 x 32 bit = 56GB / sample → 2560 x 2560 x 2160 x **1 bit** = 1.75GB / sample

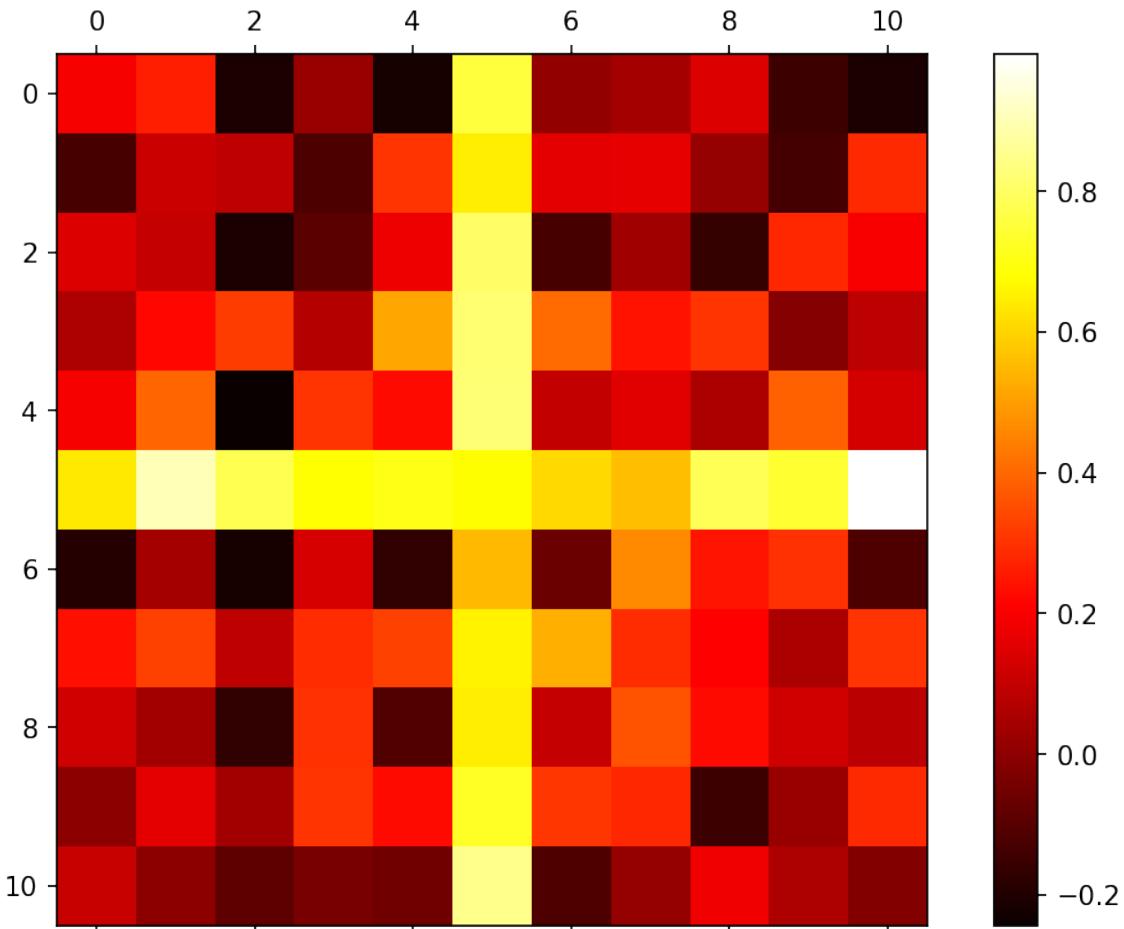
10.2 Basic segmentation: Applying a threshold to an image

Start out with a simple image of a cross with added noise $I(x, y) = f(x, y)$

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

nx = 5; ny = 5
xx, yy = np.meshgrid(np.arange(-nx, nx+1)/nx*2*np.pi,
                     np.arange(-ny, ny+1)/ny*2*np.pi)
cross_im = 1.5*np.abs(np.cos(xx*yy)) / (np.abs(xx*yy)+(3*np.pi/nx))+np.random.uniform(-
    0.25, 0.25, size = xx.shape)

fig,ax = plt.subplots(1,1,figsize=(9,6), dpi=150)
im=ax.matshow(cross_im, cmap = 'hot')
fig.colorbar(im);
```

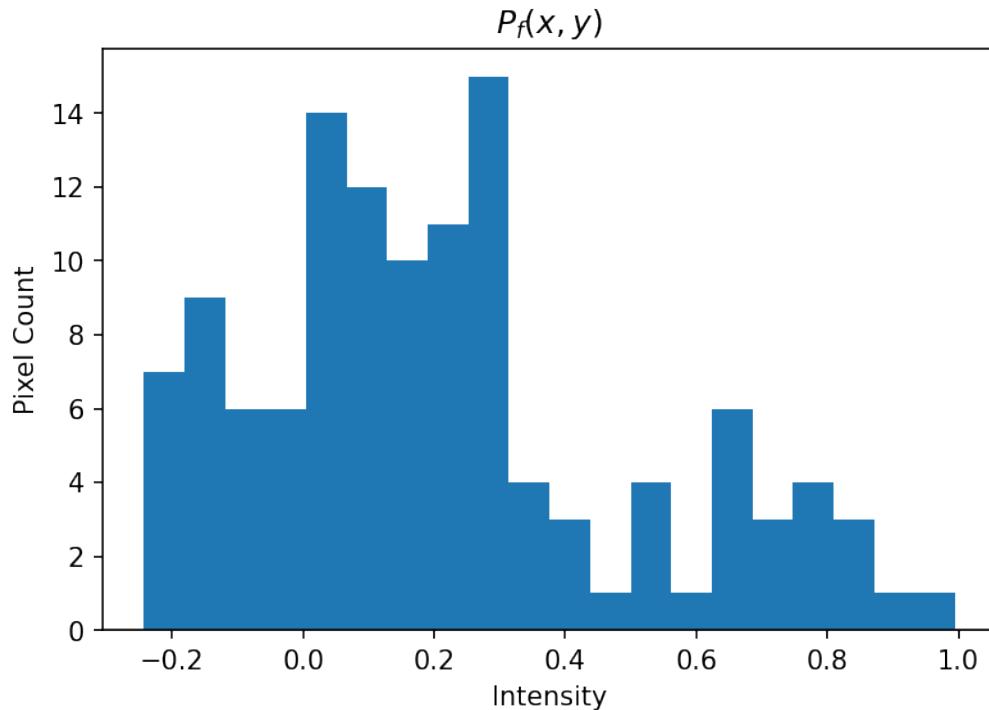


10.3 The histogram

The intensity can be described with a probability density function $P_f(x, y)$

```
fig, ax1 = plt.subplots(1,1,dpi=150)
ax1.hist(cross_im.ravel(), 20)
ax1.set_title('P_f(x,y)'); ax1.set_xlabel('Intensity'); ax1.set_ylabel('Pixel Count')

```



10.4 Applying a threshold to an image

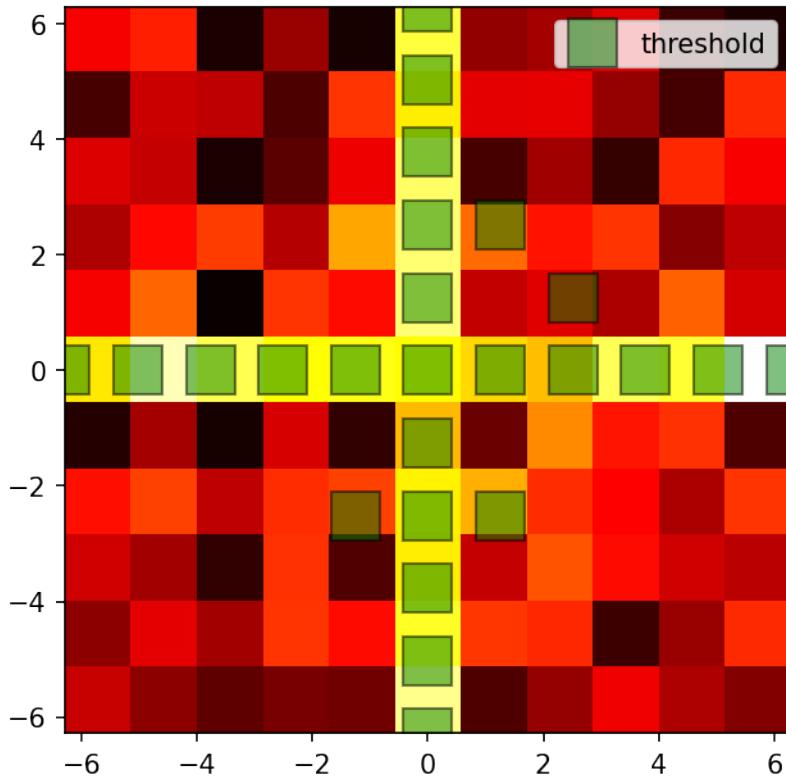
By examining the image and probability distribution function, we can *deduce* that the underlying model is a whitish phase that makes up the cross and the darkish background

Applying the threshold is a deceptively simple operation

$$I(x, y) = \begin{cases} 1, & f(x, y) \geq 0.40 \\ 0, & f(x, y) < 0.40 \end{cases}$$

```
threshold = 0.4
fig, ax1 = plt.subplots(1,1,figsize=(8,5),dpi=150)
ax1.imshow(cross_im, cmap = 'hot', extent = [xx.min(), xx.max(), yy.min(), yy.max()])
thresh_img = cross_im > threshold

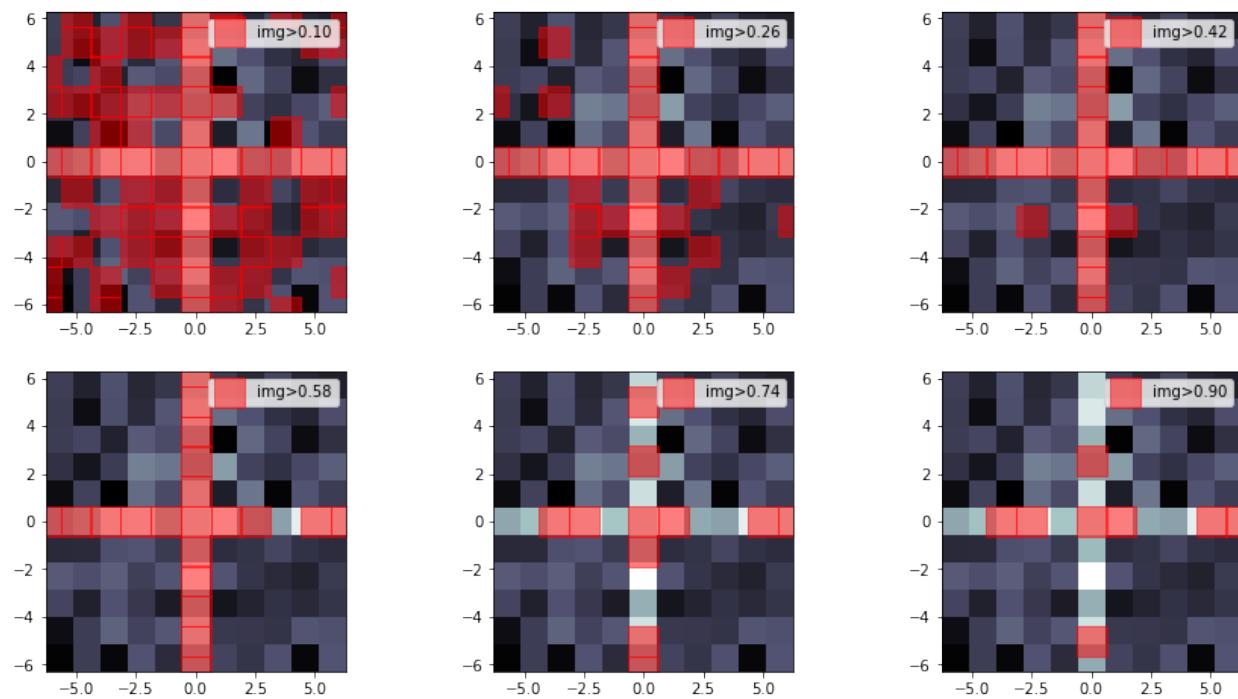
ax1.plot(xx[np.where(thresh_img)], yy[np.where(thresh_img)],
         'ks', markerfacecolor = 'green', alpha = 0.5, label = 'threshold',
         markersize = 18)
ax1.legend();
```



10.4.1 Various Thresholds

We can see the effect of choosing various thresholds

```
fig, m_axs = plt.subplots(2, 3,
                        figsize = (15, 8))
for c_thresh, ax1 in zip(np.linspace(0.1, 0.9, 6), m_axs.flatten()):
    ax1.imshow(cross_im,
               cmap = 'bone',
               extent = [xx.min(), xx.max(), yy.min(), yy.max()])
    thresh_img = cross_im > c_thresh
    ax1.plot(xx[np.where(thresh_img)], yy[np.where(thresh_img)], 'rs', alpha = 0.5,
            label = 'img>%2.2f' % c_thresh, markersize = 20)
    ax1.legend(loc = 1);
```



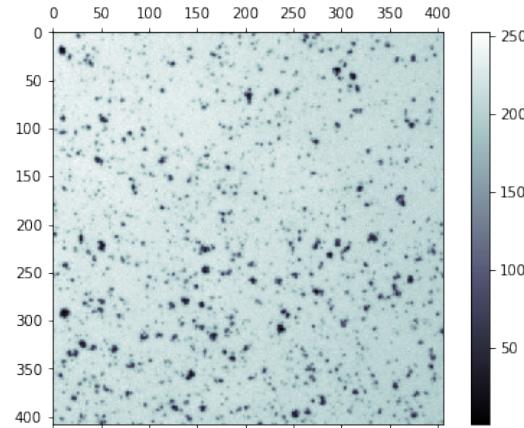
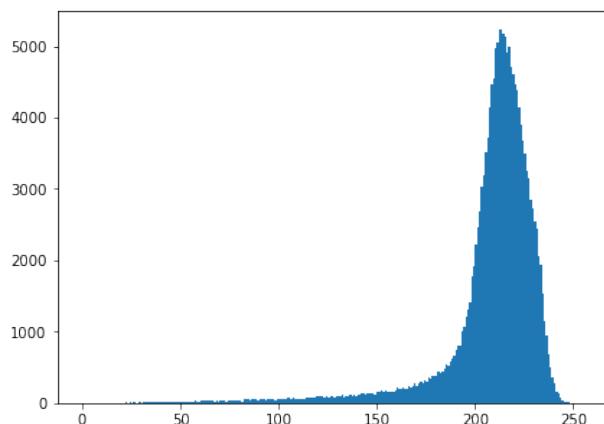
SEGMENTING CELLS

- We can perform the same sort of analysis with this image of cells
- This time we can derive the model from the basic physics of the system
- The field is illuminated by white light of nearly uniform brightness
- Cells absorb light causing darker regions to appear in the image
- *Lighter* regions have no cells
- **Darker** regions have cells

```
%matplotlib inline
from skimage.io import imread
import matplotlib.pyplot as plt
import numpy as np
```

```
cell_img = imread("figures/Cell_Colony.jpg")

fig, (ax_hist, ax_img) = plt.subplots(1, 2, figsize = (15,5))
ax_hist.hist(cell_img.ravel(), np.arange(255))
ax_obj = ax_img.matshow(cell_img, cmap = 'bone')
plt.colorbar(ax_obj);
```

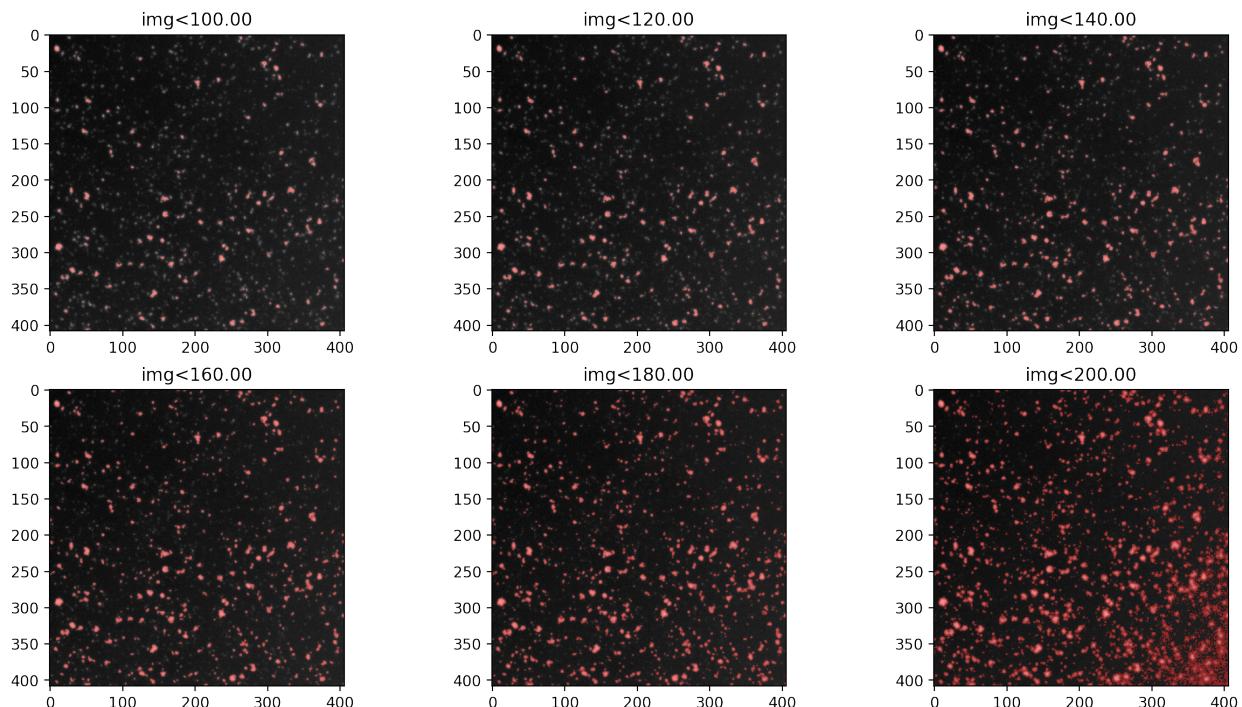


11.1 Trying different thresholds on the cell image

```
from skimage.color import label2rgb
fig, m_axs = plt.subplots(2, 3,
                        figsize = (15, 8), dpi = 200)
for c_thresh, ax1 in zip(np.linspace(100, 200, 6), m_axs.flatten()):
    thresh_img = cell_img < c_thresh

    ax1.imshow(label2rgb(thresh_img, image = 1-cell_img, bg_label = 0, alpha = 0.4))

    ax1.set_title('img<%2.2f' % c_thresh)
```



CHAPTER
TWELVE

OTHER IMAGE TYPES

While scalar images are easiest, it is possible for any type of image $I(x, y) = \vec{f}(x, y)$

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

nx = 10
ny = 10
xx, yy = np.meshgrid(np.linspace(-2*np.pi, 2*np.pi, nx),
                      np.linspace(-2*np.pi, 2*np.pi, ny))

intensity_img = 1.5*np.abs(np.cos(xx*yy)) / (np.abs(xx*yy)+(3*np.pi/nx))+np.random.
    uniform(-0.25, 0.25, size = xx.shape)

base_df = pd.DataFrame(dict(x = xx.ravel(),
                             y = yy.ravel(),
                             I_detector = intensity_img.ravel()))

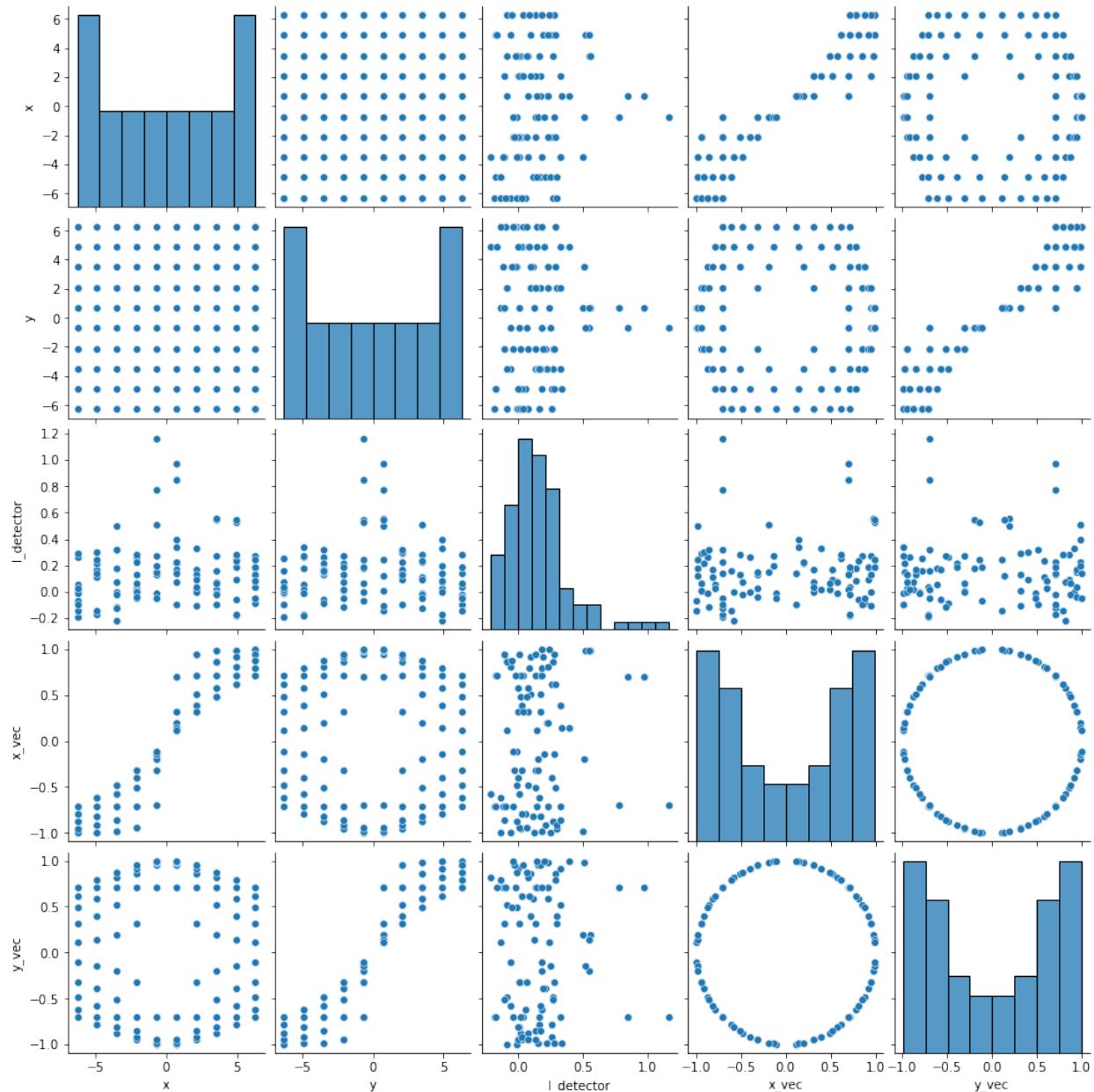
base_df['x_vec'] = base_df.apply(lambda c_row: c_row['x']/np.sqrt(1e-2+np.square(c_
    _row['x'])+np.square(c_row['y'])), 1)
base_df['y_vec'] = base_df.apply(lambda c_row: c_row['y']/np.sqrt(1e-2+np.square(c_
    _row['x'])+np.square(c_row['y'])), 1)

base_df.sample(5)
```

	x	y	I_detector	x_vec	y_vec
5	0.698132	-6.283185	-0.146359	0.110418	-0.993759
20	-6.283185	-3.490659	0.012720	-0.874073	-0.485596
77	3.490659	3.490659	-0.103736	0.706962	0.706962
0	-6.283185	-6.283185	-0.202519	-0.707062	-0.707062
47	3.490659	-0.698132	0.394822	0.980194	-0.196039

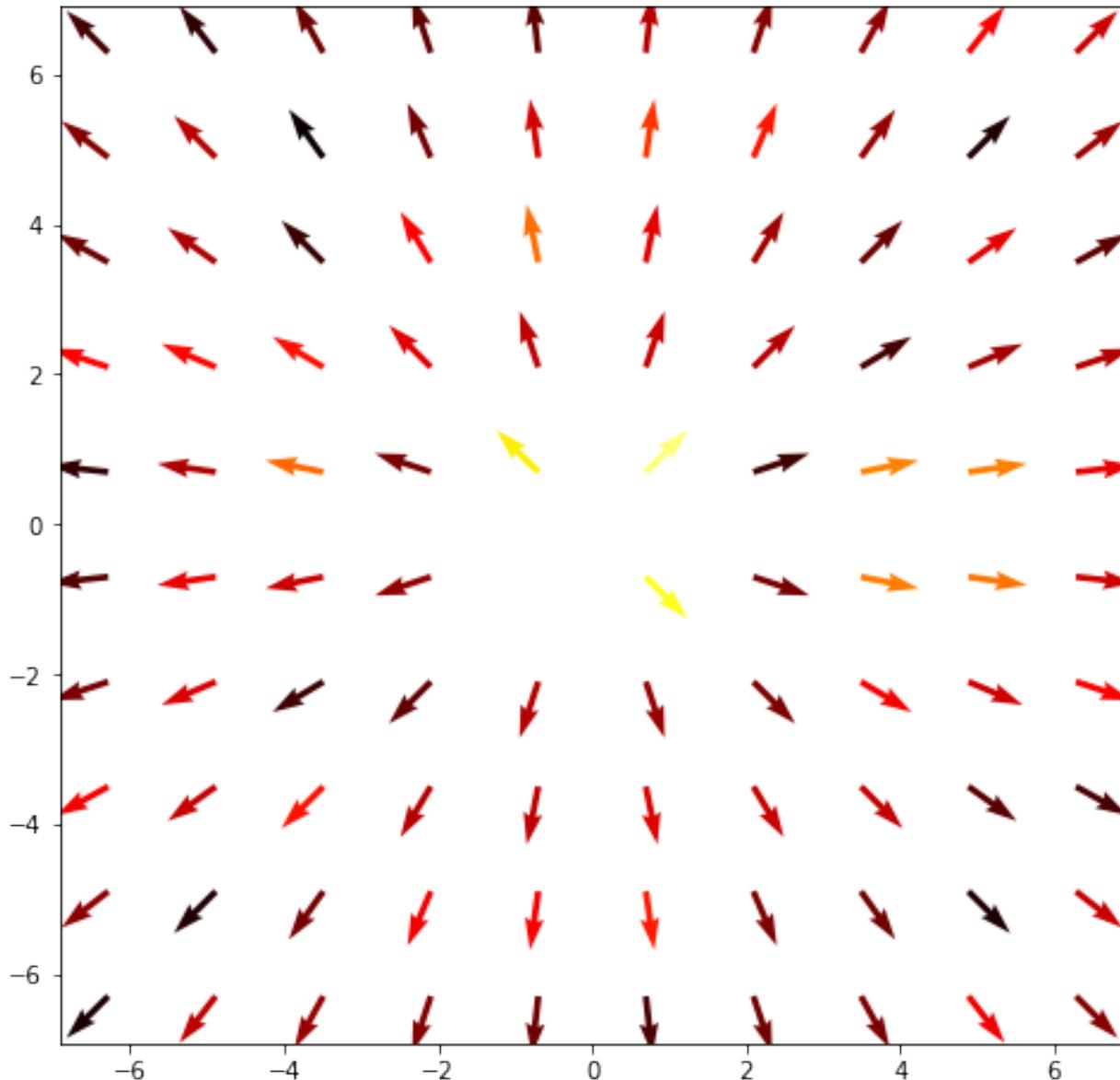
12.1 Looking at colocation histograms

```
import seaborn as sns  
sns.pairplot(base_df);
```



12.2 Vector field plot

```
fig, ax1 = plt.subplots(1,1, figsize = (8, 8))
ax1.quiver(base_df['x'], base_df['y'], base_df['x_vec'], base_df['y_vec'], base_df['I_detector'], cmap = 'hot');
```



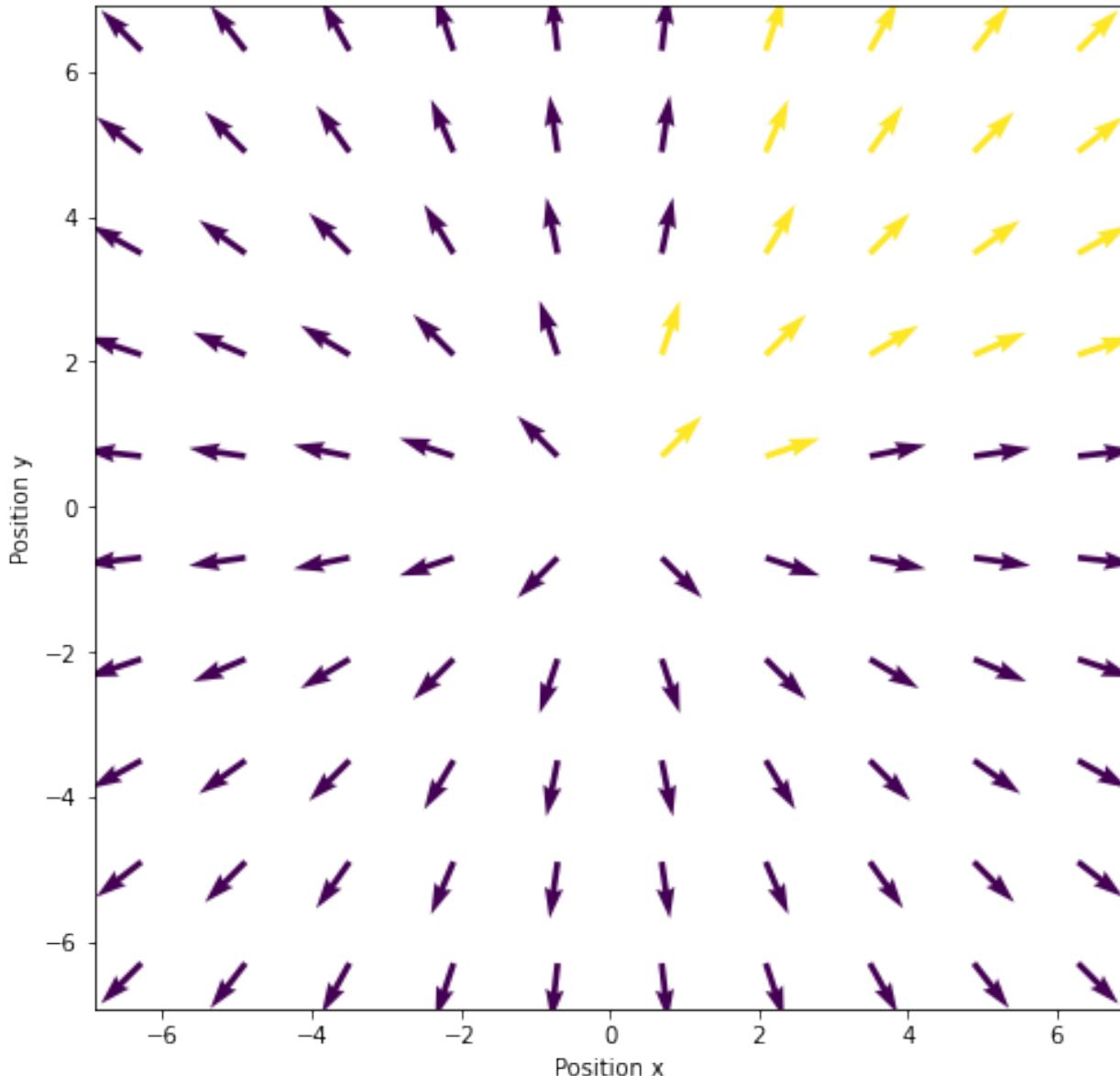
12.3 Applying a threshold to vector valued image

A threshold is now more difficult to apply since there are now two distinct variables to deal with. The standard

$$\text{approach can be applied to both } I(x, y) = \begin{cases} 1, & \vec{f}_x(x, y) \geq 0.25 \text{ and} \\ & \vec{f}_y(x, y) \geq 0.25 \\ 0, & \text{otherwise} \end{cases}$$

```
thresh_df = base_df.copy()
thresh_df['thresh'] = thresh_df.apply(lambda c_row: c_row['x_vec']>0.25 and c_row['y_
→vec']>0.25, 1)

fig, ax1 = plt.subplots(1,1, figsize = (8, 8))
ax1.quiver(thresh_df['x'], thresh_df['y'], thresh_df['x_vec'], thresh_df['y_vec'], u
→thresh_df['thresh']);
ax1.set_xlabel('Position x'); ax1.set_ylabel('Position y');
```

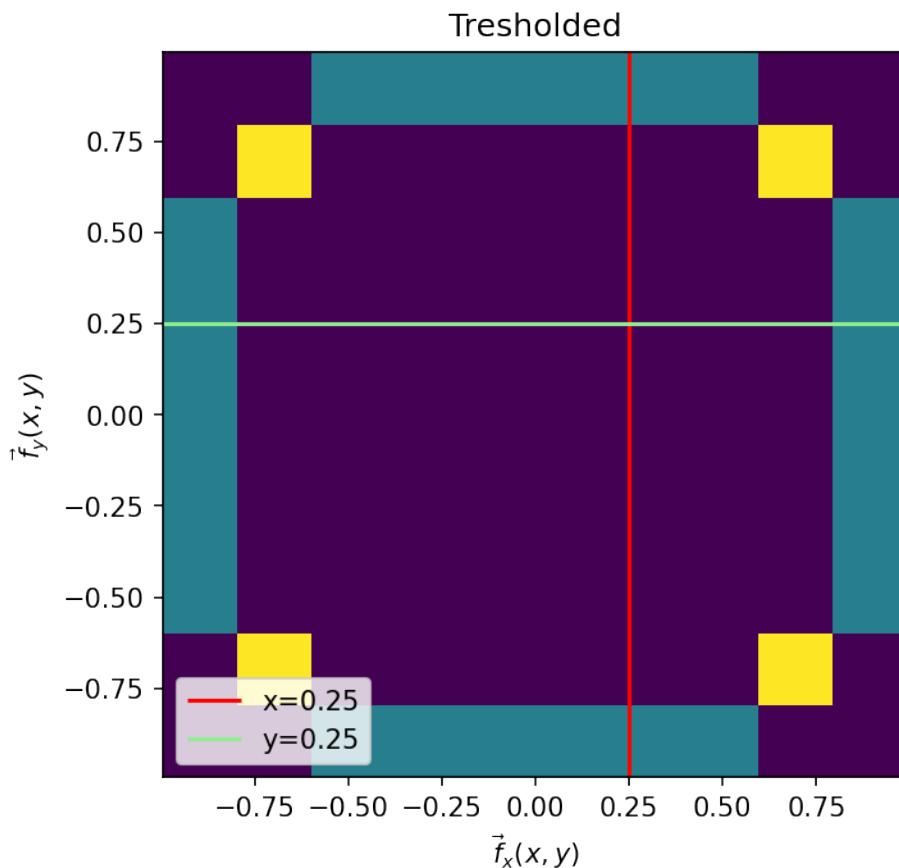


12.3.1 Histogram of the vectors

This can also be shown on the joint probability distribution as

```
fig, ax = plt.subplots(1,1, figsize = (5, 5), dpi = 150)
ax.hist2d(thresh_df['x_vec'], thresh_df['y_vec'], cmap = 'viridis'); ax.set_title(
    'Thresholded');
ax.set_xlabel('$\vec{f}_x(x,y)$'); ax.set_ylabel('$\vec{f}_y(x,y)$');
ax.vlines(0.25,ymin=-1,ymax=1,color='red',label='x=0.25');ax.hlines(0.25,xmin=-1,
    xmax=1,color='lightgreen', label='y=0.25');ax.legend(loc='lower left')
```

<matplotlib.legend.Legend at 0x7ffdeabb070>



12.3.2 Applying a threshold

Given the presence of two variables; however, more advanced approaches can also be investigated. For example we can keep only components parallel to the x axis by using the dot product. $I(x, y) = \begin{cases} 1, & |\vec{f}(x, y) \cdot \vec{i}| = 1 \\ 0, & \text{otherwise} \end{cases}$

12.3.3 Thresholding orientations

We can tune the angular acceptance by using the fact $\vec{x} \cdot \vec{y} = |\vec{x}||\vec{y}| \cos(\theta_{x \rightarrow y}) < br/ >< br/ > I(x, y) = \begin{cases} 1, & \cos^{-1}(\vec{f}(x, y) \cdot \vec{i}) \leq \theta^\circ \\ 0, & \text{otherwise} \end{cases}$

Basic Segmentation and Discrete Binary Structures

Quantitative Big Imaging ETHZ: 227-0966-00L

Part 2

A Machine Learning Approach to Image Processing

Segmentation and all the steps leading up to it are really a specialized type of learning problem.

Let's look at an important problem for electron microscopy imaging...

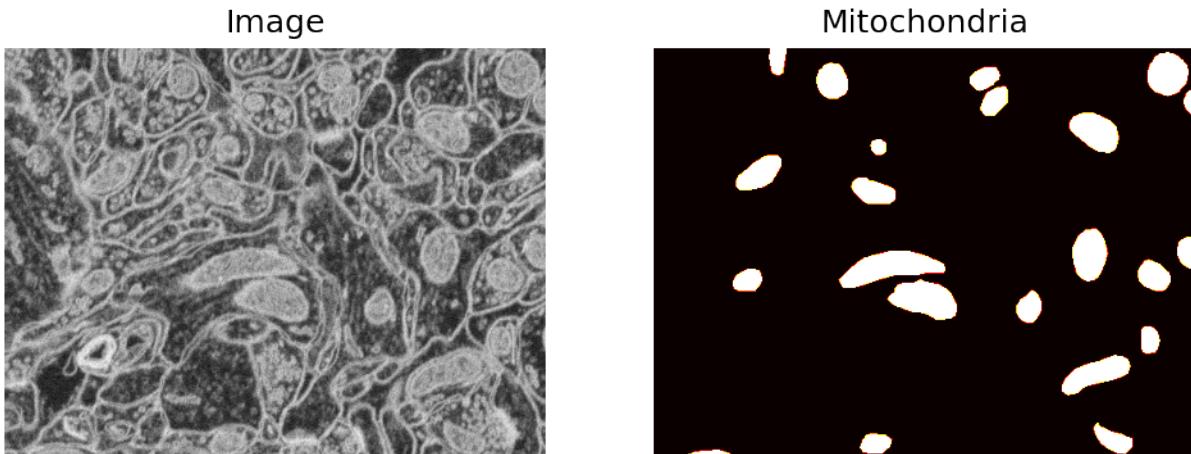
```
import numpy as np
import matplotlib.pyplot as plt
from skimage.color import rgb2gray
from skimage.io import imread
%matplotlib inline
```

```
-----
ModuleNotFoundError                       Traceback (most recent call last)
<ipython-input-1-33a394319bb8> in <module>
      1 import numpy as np
      2 import matplotlib.pyplot as plt
----> 3 from skimage.color import rgb2gray
      4 from skimage.io import imread
      5 get_ipython().run_line_magic('matplotlib', 'inline')

ModuleNotFoundError: No module named 'skimage'
```

```
cell_img = (255-imread("data/em_image.png")[:, :, ::2])/255.0
cell_seg = imread("data/em_image_seg.png")[:, :, ::2]>0

fig, (ax1, ax2) = plt.subplots(1, 2, figsize=(8, 4), dpi=150)
ax1.imshow(cell_img, cmap='gray'); ax1.set_title('Image');           ax1.axis('off');
ax2.imshow(cell_seg, cmap='hot'); ax2.set_title('Mitochondria'); ax2.axis('off');
```



We want to identify which class each pixel belongs to.

What does identify mean?

- Classify the pixels in a mitochondria as *Foreground*
- Classify the pixels outside of a mitochondria as *Background*

How do we quantify this?

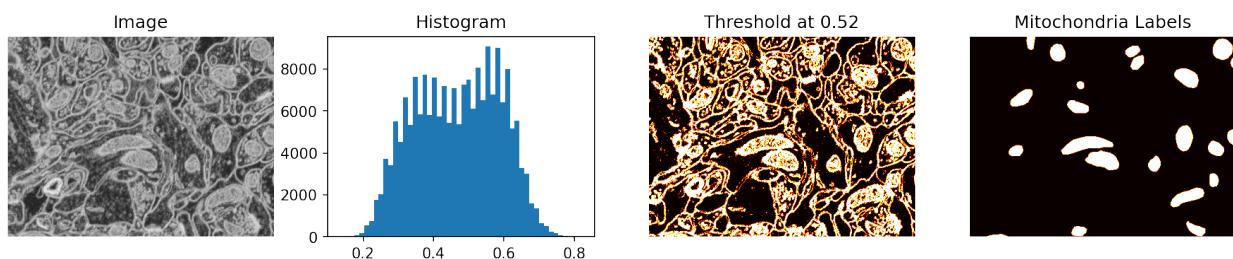
- **True Positive** values in the mitochondria that are classified as *Foreground*
- **True Negative** values outside the mitochondria that are classified as *Background*
- **False Positive** values outside the mitochondria that are classified as *Foreground*
- **False Negative** values in the mitochondria that are classified as *Background*

```
fig, ax = plt.subplots(1, 4, figsize=(15, 2.5), dpi=150)

ax[0].imshow(cell_img, cmap='gray'); ax[0].set_title('Image'); ax[0].axis('off')
ax[1].hist(cell_img.ravel(), bins=50); ax[1].set_title('Histogram')

thresh      = 0.52
thresh_img = cell_img > thresh # Apply a single threshold

ax[2].imshow(thresh_img, cmap='hot'); ax[2].set_title('Threshold at {}'.format(thresh)); ax[2].axis('off')
ax[3].imshow(cell_seg,   cmap='hot'); ax[3].set_title('Mitochondria Labels'); ax[3].axis('off');
```



Check the performance of the thresholding

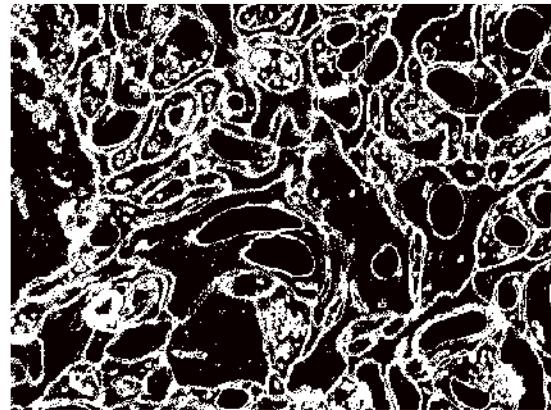
```
# Support function for the plot labels
def tp_func(real_img_idx, pred_img_idx):
    if real_img_idx == 1 and pred_img_idx == 1:
        return 'True Positive', 'green'
    if real_img_idx == 0 and pred_img_idx == 0:
        return 'True Negative', 'green'
    if real_img_idx == 0 and pred_img_idx == 1:
        return 'False Positive', 'red'
    if real_img_idx == 1 and pred_img_idx == 0:
        return 'False Negative', 'red'

out_results = {}
fig, m_ax = plt.subplots(2, 2, figsize=(8, 7), dpi=150)
for real_img_idx, n_ax in zip([0, 1], m_ax):
    for pred_img_idx, c_ax in zip([0, 1], n_ax):
        match_img = (thresh_img == pred_img_idx) & (cell_seg == real_img_idx)
        (tp_title, color) = tp_func(real_img_idx, pred_img_idx)
        c_ax.matshow(match_img, cmap='hot')
        out_results[tp_title] = np.sum(match_img)
        c_ax.set_title("{0} ({1})".format(tp_title, out_results[tp_title]), color=color)
        c_ax.axis('off')
```

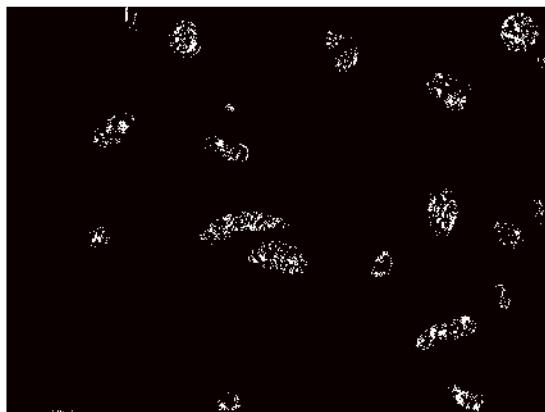
True Negative (118050)



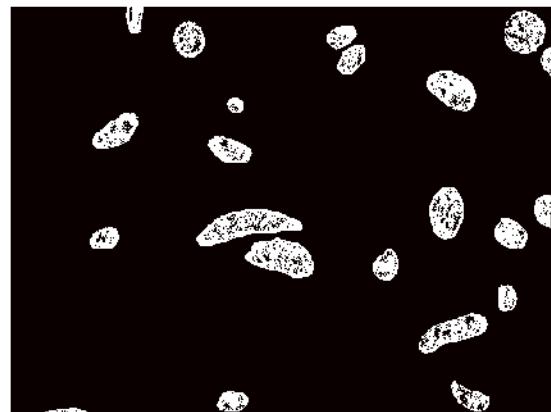
False Positive (61945)



False Negative (2932)



True Positive (13681)



Apply Precision and Recall

- **Recall** (sensitivity) $\frac{TP}{TP+FN}$
- **Precision** $\frac{TP}{TP+FP}$

```
print('Recall: {:.2f}'.format(out_results['True Positive'] /
                             (out_results['True Positive']+out_results['False Negative']
                             ↵)))
print('Precision: {:.2f}'.format(out_results['True Positive'] /
                                 (out_results['True Positive']+out_results['False Positive'
                                 ↵])))
```

```
Recall: 0.82
Precision: 0.18
```

The confusion matrix (revisited)

Confusion matrix

ROC Curve

Reciever Operating Characteristic (first developed for WW2 soldiers detecting objects in battlefields using radar).

The ideal is the top-right (identify everything and miss nothing).

As we saw before, for a single threshold value 0.5, we were able to compute a single recall and precision.

If we want to make an ROC curve we take a number of threshold values

```
import pandas as pd
from collections import OrderedDict

out_vals = []
for thresh_val in np.linspace(0.1, 0.9):
    thresh_img = cell_img > thresh_val
    for real_img_idx in [0, 1]:
        for pred_img_idx in [0, 1]:
            match_img = (thresh_img == pred_img_idx) & (
                cell_seg == real_img_idx)
            tp_title = tp_func(real_img_idx, pred_img_idx)
            out_results[tp_title] = np.sum(match_img)
    out_vals += [
        OrderedDict(
            Threshold=thresh_val,
            Recall=out_results['True Positive'] /
            (out_results['True Positive']+out_results['False Negative']),
            Precision=(out_results['True Positive'] /
            (out_results['True Positive']+out_results['False Positive'])),
            False_Positive_Rate=(out_results['False Positive'] /
            (out_results['False Positive']+out_results['True Negative'])),
            **out_results
        )
    ]

roc_df = pd.DataFrame(out_vals)
roc_df.head(3)
```

	Threshold	Recall	Precision	False_Positive_Rate	True_Negative	\
0	0.100000	0.823512	0.180903	0.344148	118050	
1	0.116327	0.823512	0.180903	0.344148	118050	
2	0.132653	0.823512	0.180903	0.344148	118050	
	False_Positive	False_Negative	True_Positive	(True_Negative, green)	(True_Negative, green)	\
0	61945	2932	13681		0	
1	61945	2932	13681		0	
2	61945	2932	13681		0	
	(False_Positive, red)	(False_Negative, red)	(True_Positive, green)	(True_Positive, green)	(True_Positive, green)	
0	179995		0	0	16613	
1	179995		0	0	16613	
2	179995		0	0	16613	

Making ROC Curves Easier

ROC curves are a very common tool for analyzing the performance of binary classification systems and there are a large number of tools which can automatically make them. Here we show how it is done with scikit-image.

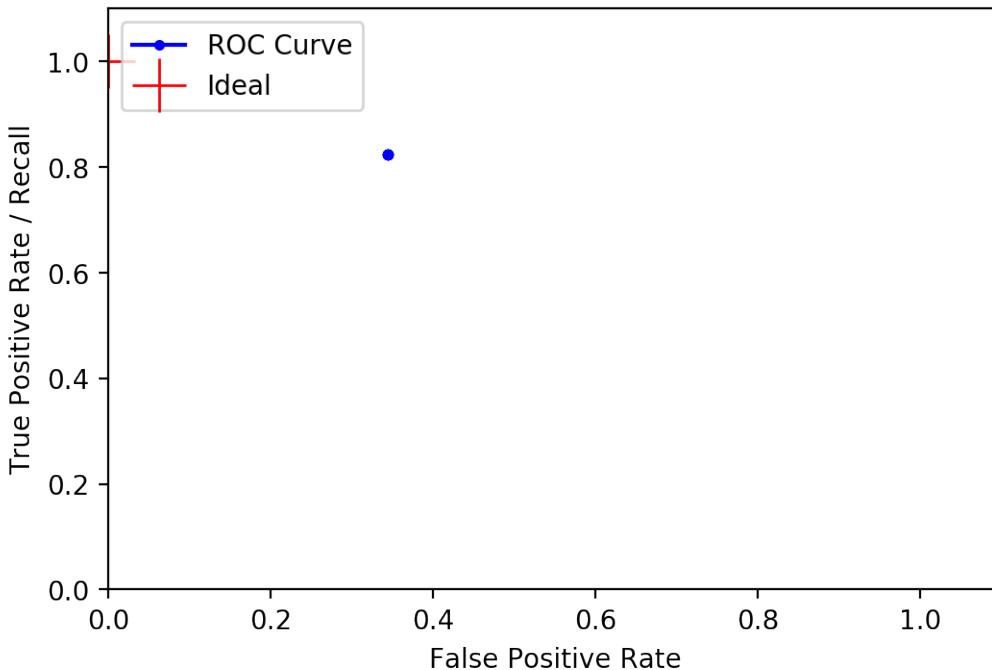
Another way of showing the ROC curve (more common for machine learning rather than medical diagnosis) is using the True positive rate and False positive rate

- **True Positive Rate** (recall)= $TP/(TP + FN)$
- **False Positive Rate** = $FP/(FP + TN)$

These show very similar information with the major difference being the goal is to be in the upper left-hand corner. Additionally random guesses can be shown as the slope 1 line. Therefore for a system to be useful it must lie above the random line.

```
fig, ax1 = plt.subplots(1, 1, dpi=200)
ax1.plot(roc_df['False_Positive_Rate'], roc_df['Recall'], 'b.-', label='ROC Curve')
ax1.plot(0, 1.0, 'r+', markersize=20, label='Ideal')
ax1.set_xlim(0, 1.1)
ax1.set_ylim(0, 1.1)
ax1.set_ylabel('True Positive Rate / Recall')
ax1.set_xlabel('False Positive Rate')
ax1.legend(loc=2)
```

<matplotlib.legend.Legend at 0x1c181c51d0>



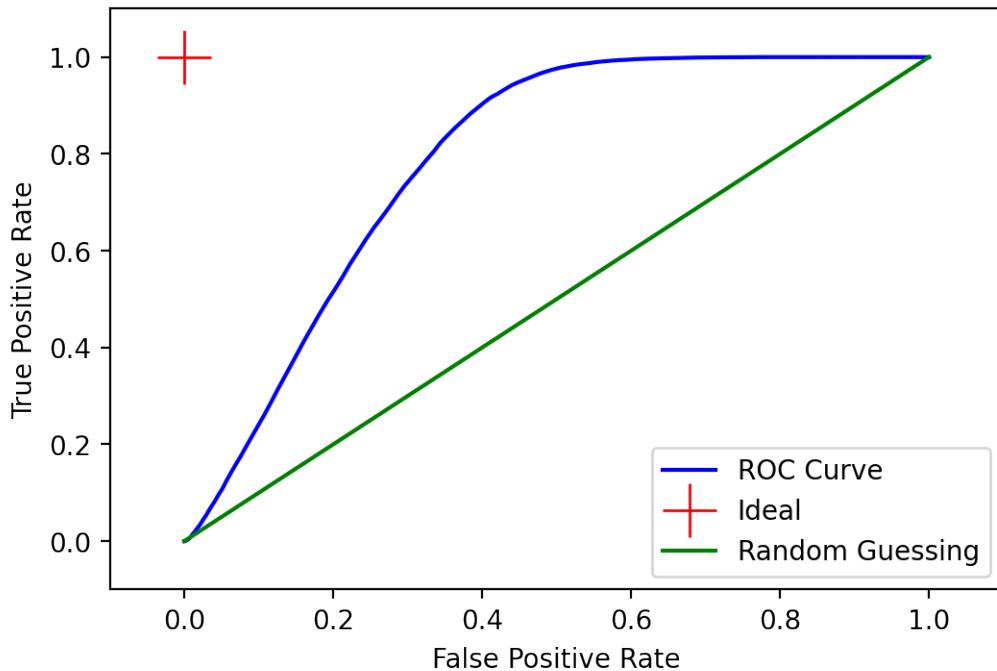
```
from sklearn.metrics import roc_curve
fpr, tpr, thresholds = roc_curve(cell_seg.ravel().astype(int),
                                  cell_img.ravel())

fig, ax1 = plt.subplots(1, 1, dpi=200)
ax1.plot(fpr, tpr, 'b.-', markersize=0.01, label='ROC Curve')
```

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```
ax1.plot(0.0, 1.0, 'r+', markersize=20, label='Ideal')
ax1.plot([0, 1], [0, 1], 'g-', label='Random Guessing')
ax1.set_xlim(-0.1, 1.1)
ax1.set_ylim(-0.1, 1.1)
ax1.set_xlabel('False Positive Rate')
ax1.set_ylabel('True Positive Rate')
ax1.legend(loc=0);
```



```
from skimage.filters import gaussian, median

def no_filter(x):
    return x

def gaussian_filter(x):
    return gaussian(x, sigma=2)

def diff_of_gaussian_filter(x):
    return -gaussian(x, sigma=3)

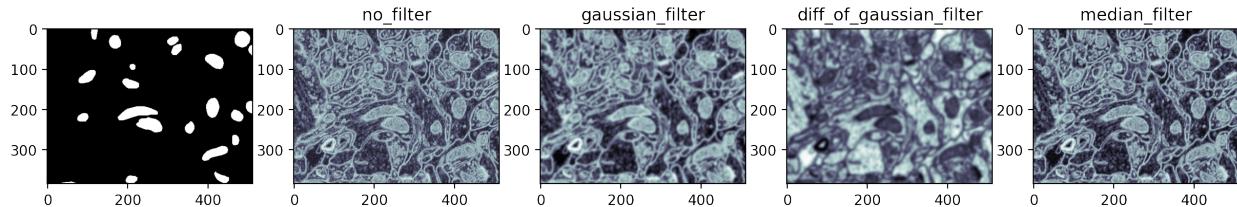
def median_filter(x):
    return median(x, np.ones((3, 3)))

fig, m_axs = plt.subplots(1, 5, figsize=(15, 3), dpi=200)
m_axs[0].imshow(cell_seg, cmap='gray')
for c_filt, c_ax in zip([no_filter, gaussian_filter, diff_of_gaussian_filter, median_filter], m_axs[1:]):
    c_ax.imshow(c_filt(cell_img), cmap='bone')
```

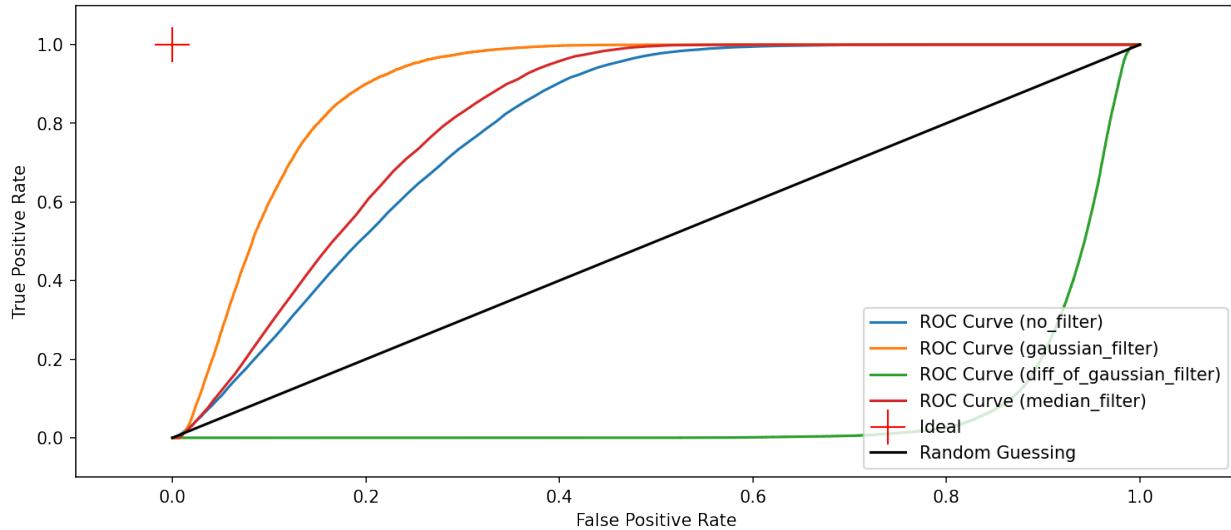
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```
c_ax.set_title(c_filt.__name__)
```



```
fig, ax1 = plt.subplots(1, 1, figsize=(12, 5), dpi=150)
for c_filt in [no_filter, gaussian_filter, diff_of_gaussian_filter, median_filter]:
    fpr, tpr, thresholds = roc_curve(cell_seg.ravel().astype(int),
                                      c_filt(cell_img).ravel())
    ax1.plot(fpr, tpr, '--', markersize=0.01,
              label='ROC Curve ({})'.format(c_filt.__name__))
ax1.plot(0.0, 1.0, 'r+', markersize=20, label='Ideal')
ax1.plot([0, 1], [0, 1], 'k-', label='Random Guessing')
ax1.set_xlim(-0.1, 1.1)
ax1.set_ylim(-0.1, 1.1)
ax1.set_xlabel('False Positive Rate')
ax1.set_ylabel('True Positive Rate')
ax1.legend(loc="lower right", fontsize=10);
```



We can then use this ROC curve to compare different filters (or even entire workflows), if the area is higher the approach is better.

Different approaches can be compared by area under the curve

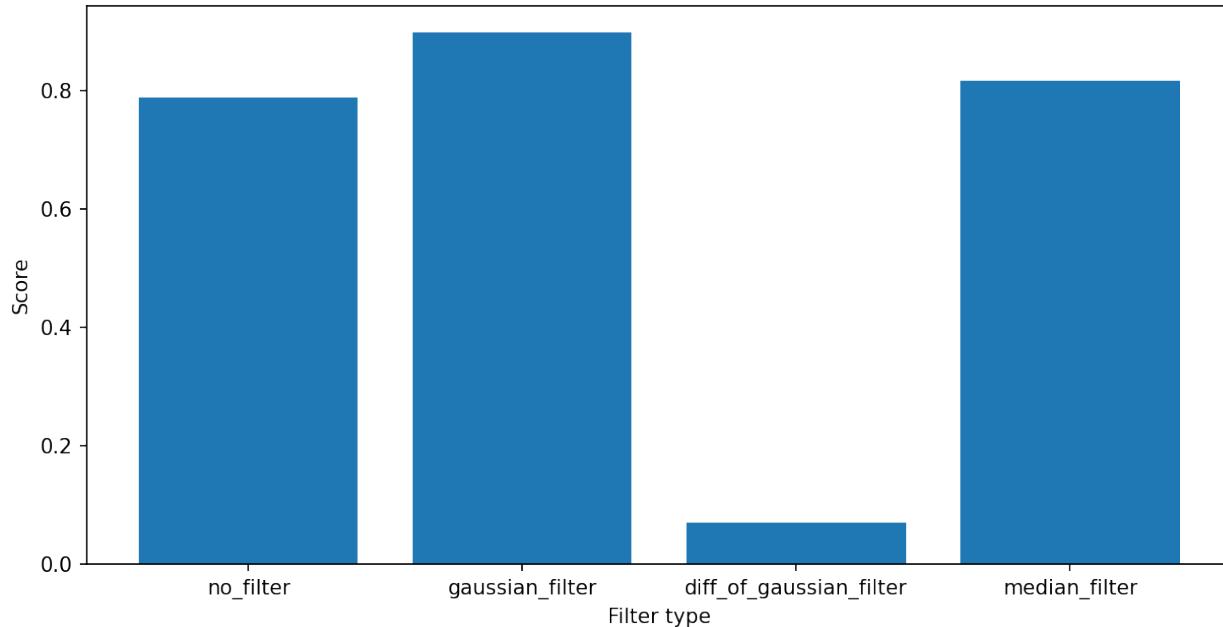
```
from sklearn.metrics import roc_auc_score
scores = []
names = ['no_filter', 'gaussian_filter', 'diff_of_gaussian_filter', 'median_filter']
for c_filt in [no_filter, gaussian_filter, diff_of_gaussian_filter, median_filter]:
    scores.append(roc_auc_score(cell_seg.ravel().astype(int), c_filt(cell_img).
                                ravel()))
#     print('%s - %.2f' % (c_filt.__name__, roc_auc_score(cell_seg.ravel().
#                                astype(int)),
```

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```
#                                     c_filt(cell_img).ravel())))

plt.figure(figsize=[10,5],dpi=150)
plt.bar(names,scores); plt.xlabel('Filter type'),plt.ylabel('Score');
```



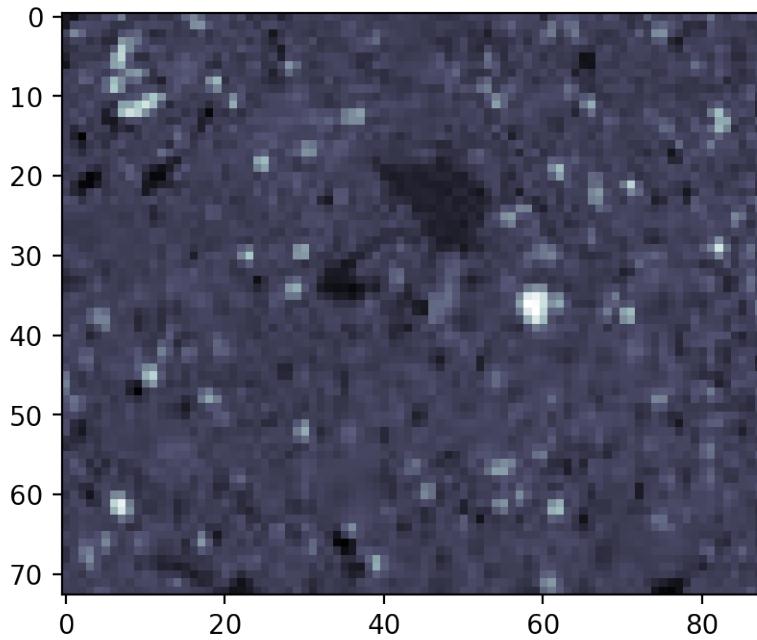
Evaluating Models

- <https://github.com/jvns/talks/blob/master/pydatanyc2014/slides.md>
- <http://mathbabe.org/2012/03/06/the-value-added-teacher-model-sucks/>

Multiple Phases: Segmenting Shale

- Shale provided from Kanitpanyacharoen, W. (2012). Synchrotron X-ray Applications Toward an Understanding of Elastic Anisotropy.
- Here we have a shale sample measured with X-ray tomography with three different phases inside (clay, rock, and air).
- The model is that because the chemical composition and density of each phase is different they will absorb different amounts of x-rays and appear as different brightnesses in the image

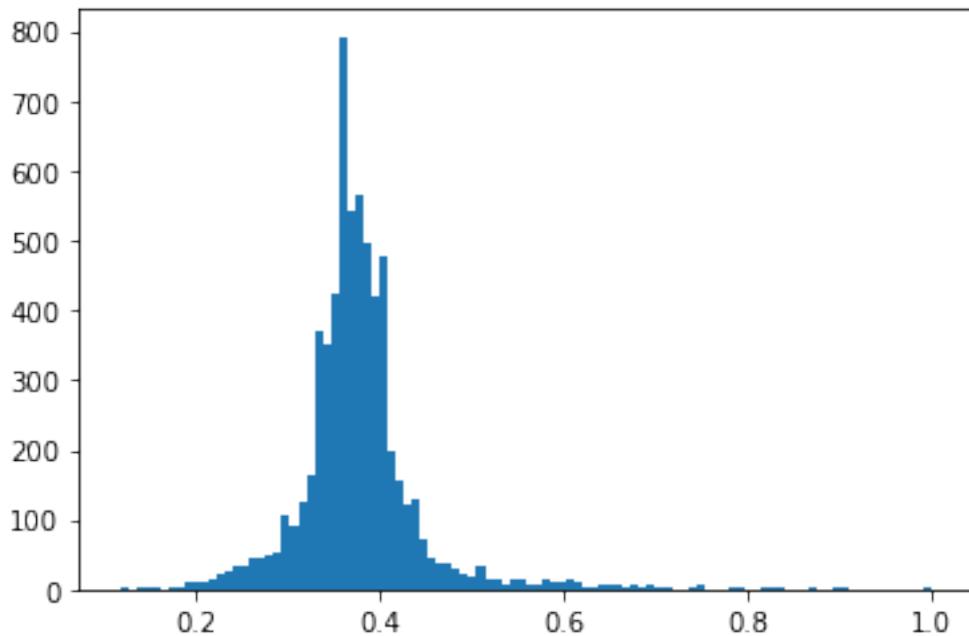
```
import numpy as np
import matplotlib.pyplot as plt
from skimage.color import rgb2gray
from skimage.io import imread
%matplotlib inline
shale_img = imread("figures/ShaleSample.jpg")/255.0
fig, ax1 = plt.subplots(1, 1, dpi=200)
ax1.imshow(shale_img, cmap='bone');
```



Ideally we would derive 3 values for the thresholds based on a model for the composition of each phase and how much it absorbs, but that is not always possible or practical.

- While there are 3 phases clearly visible in the image, the histogram is less telling (even after being re-scaled).

```
plt.hist(shale_img.ravel(), 100);
```

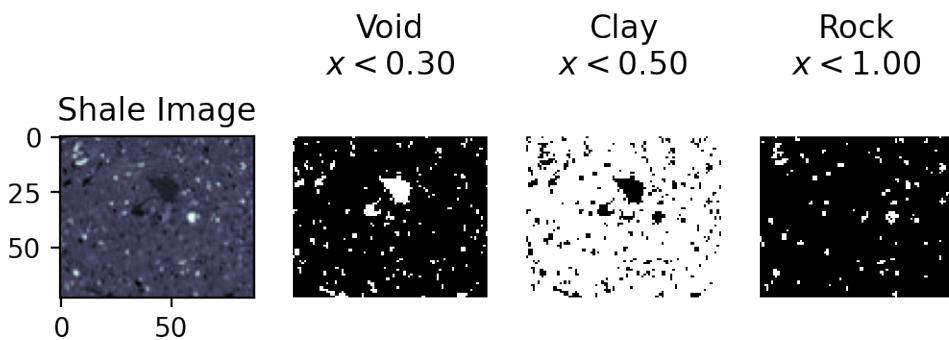


Multiple Segmentations

For this exercise we choose arbitrarily 3 ranges for the different phases and perform visual inspection

The relation can explicitly be written out as $I(x) = \begin{cases} \text{Void}, & 0 \leq x \leq 0.3 \\ \text{Clay}, & 0.3 < x \leq 0.5 \\ \text{Rock}, & 0.5 < x \end{cases}$

```
fig, m_axs = plt.subplots(1, 4, dpi=200, figsize=(6, 3))
m_axs[0].imshow(shale_img, cmap='bone')
m_axs[0].set_title('Shale Image')
used_vox = np.zeros_like(shale_img).astype(np.uint8)
for c_ax, c_max, c_title in zip(m_axs[1:], [0.3, 0.5, 1.0], ['Void', 'Clay', 'Rock']):
    c_slice = (shale_img < c_max)-used_vox
    c_ax.matshow(c_slice, cmap='bone')
    used_vox += c_slice
    c_ax.axis('off')
    c_ax.set_title('%s\nx<%2.2f' % (c_title, c_max))
```



Implementation

The implementations of basic thresholds and segmentations is very easy since it is a unary operation of a single image $f(I(\bar{x}))$. In mathematical terms this is called a map and since it does not require information from neighboring voxels or images it can be calculated for each point independently (*parallel*). Filters on the other hand almost always depend on neighboring voxels and thus the calculations are not as easy to separate.

Implementation Code

Matlab / Python (numpy)

The simplest is a single threshold in Matlab:

```
thresh_img = gray_img > thresh
```

A more complicated threshold:

```
thresh_img = (gray_img > thresh_a) & (gray_img < thresh_b)
```

Python

```
thresh_img = map(lambda gray_val: gray_val>thresh,
                 gray_img)
```

Java

```
boolean[] thresh_img = new boolean[x_size*y_size*z_size];
for(int x=x_min;x<x_max;x++)
    for(int y=y_min;y<y_max;y++)
        for(int z=z_min;z<z_max;z++) {
            int offset=(z*y_size+y)*x_size+x;
            thresh_img[offset]=gray_img[offset]>thresh;
        }
    }
```

In C/C++

```
bool* thresh_img = malloc(x_size*y_size*z_size * sizeof (bool));

for(int x=x_min;x<x_max;x++)
    for(int y=y_min;y<y_max;y++)
        for(int z=z_min;z<z_max;z++) {
            int offset=(z*y_size+y)*x_size+x;
            thresh_img[offset]=gray_img[offset]>thresh;
        }
    }
```

Morphology

We can now utilize information from neighborhood voxels to improve the results. These steps are called morphological operations. We return to the original image of a cross

Like filtering the assumption behind morphological operations are

- nearby voxels in **real** images are related / strongly correlated with one another
- noise and imaging artifacts are less spatially correlated.

Therefore these imaging problems can be alleviated by adjusting the balance between local and neighborhood values.

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

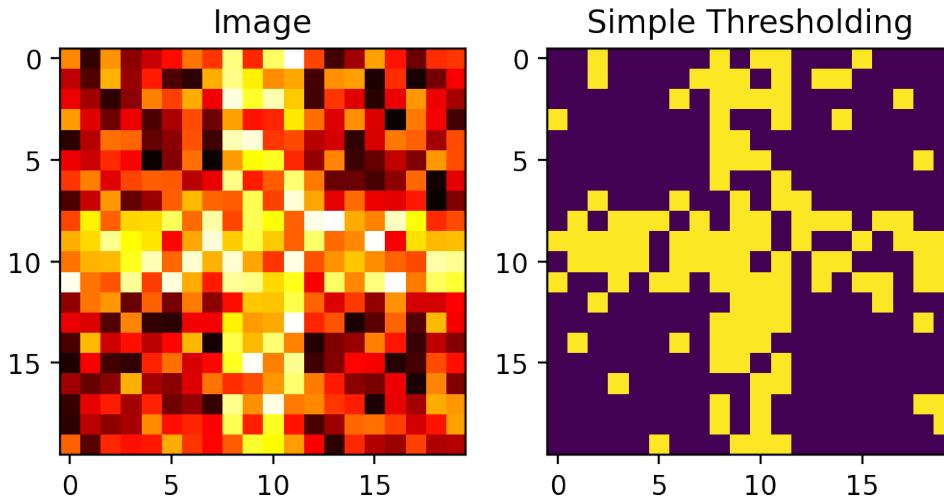
nx = 20
ny = 20
xx, yy = np.meshgrid(np.linspace(-10, 10, nx),
                      np.linspace(-10, 10, ny))
np.random.seed(2018)
cross_im = 1.1*((np.abs(xx) < 2)+(np.abs(yy) < 2)) + \
           np.random.uniform(-1.0, 1.0, size=xx.shape)
fig, (ax1, ax2) = plt.subplots(1, 2, figsize=(6, 3.5), dpi=200)
```

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```
ax1.imshow(cross_im, cmap='hot')
ax1.set_title('Image')
ax2.imshow(cross_im > 0.8)
ax2.set_title('Simple Thresholding')
```

```
Text(0.5, 1.0, 'Simple Thresholding')
```



Fundamentals: Neighborhood

A neighborhood consists of the pixels or voxels which are of sufficient proximity to a given point. There are a number of possible definitions which largely affect the result when it is invoked.

- A large neighborhood performs operations over larger areas / volumes
- Computationally intensive
- Can *smooth* out features
- A small neighborhood performs operations over small areas / volumes
- Computationally cheaper
- Struggles with large noise / filling large holes

The neighborhood is important for a large number of image and other (communication, mapping, networking) processing operations:

- filtering
- morphological operations
- component labeling
- distance maps
- image correlation based tracking methods

It is often called structuring element (or `selem` for sort / code), but has exactly the same meaning

Fundamentals: Neighbors in 2D

For standard image operations there are two definitions of neighborhood. The 4 and 8 adjacent neighbors shown below. Given the blue pixel in the center the red are the 4-adjacent and the red and green make up the 8 adjacent. We expand beyond this to disk, cross, vertical and horizontal lines

```
from skimage.morphology import disk, octagon as oct_func, star

def h_line(n):
    return np.pad(np.ones((1, 2*n+1)), [[n, n], [0, 0]], mode='constant', constant_
    values=0).astype(int)

def v_line(n):
    return h_line(n).T

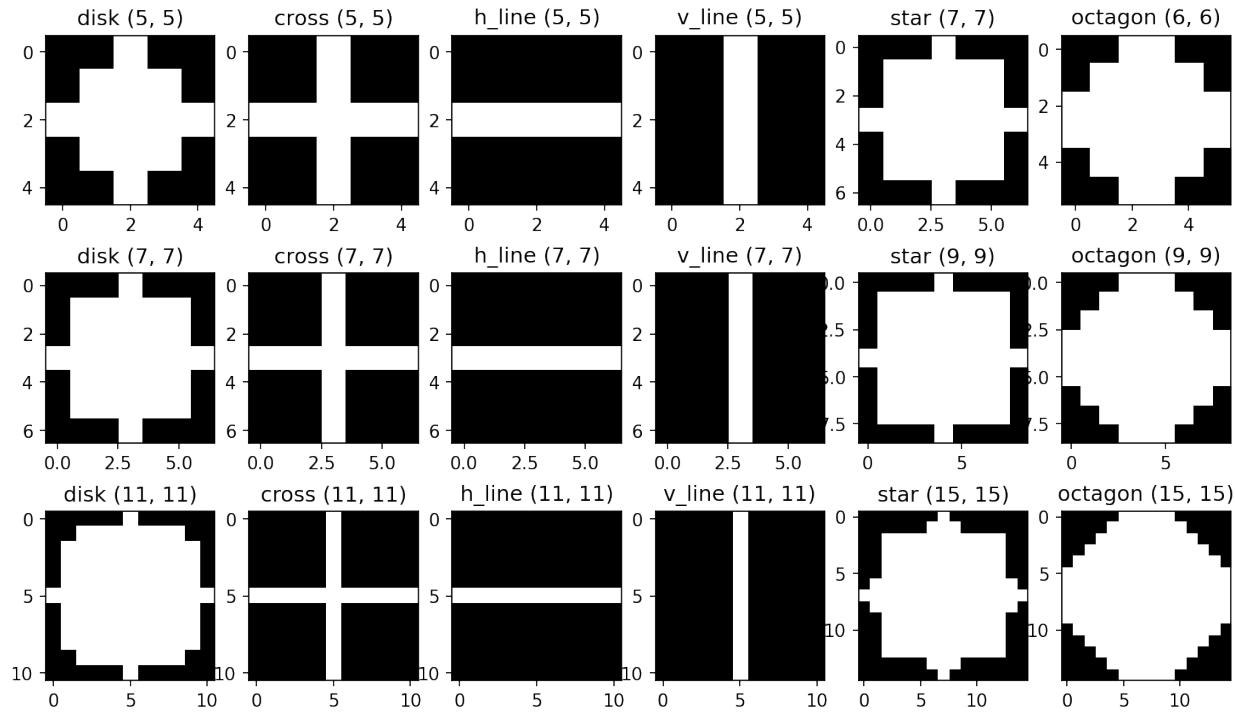
def cross(n):
    return ((h_line(n)+v_line(n)) > 0).astype(int)

def octagon(n):
    return oct_func(n, n)
```

```
neighbor_functions = [disk, cross, h_line, v_line, star, octagon]
sizes = [2, 3, 5]
fig, m_axs = plt.subplots(len(sizes), len(neighbor_functions),
                         figsize=(12, 7), dpi=150)
for c_dim, c_axs in zip(sizes, m_axs):
    for c_func, c_ax in zip(neighbor_functions, c_axs):
        c_ax.imshow(c_func(c_dim), cmap='bone', interpolation='none')
        c_ax.set_title('{} {}'.format(c_func.__name__, c_func(c_dim).shape))

plt.suptitle('Different neighborhood shapes and sizes', fontsize=20);
```

Different neighborhood shapes and sizes



Erosion and Dilation

Erosion

If any of the voxels in the neighborhood are 0/false than the voxel will be set to 0

- Has the effect of peeling the surface layer off of an object
-

Dilation

If any of the voxels in the neighborhood are 1/true then the voxel will be set to 1

- Has the effect of adding a layer onto an object (dunking an strawberry in chocolate, adding a coat of paint to a car)

Applied Erosion and Dilation

```
import numpy as np
import matplotlib.pyplot as plt
import skimage.morphology as morph

img=np.load('data/morphimage.npy')

oimg=morph.opening(img,np.array([[0,1,0],[1,1,1],[0,1,0]]))
cimg=morph.closing(img,np.array([[0,1,0],[1,1,1],[0,1,0]]))
s=255.0
cmap = [[230/s,230/s,230/s],
         [255/s,176/s,159/s],
         [0.0/s,0.0/s,0.0/s]]
```

Dilation

We can use dilation to expand objects, for example a too-low threshold value leading to disconnected components

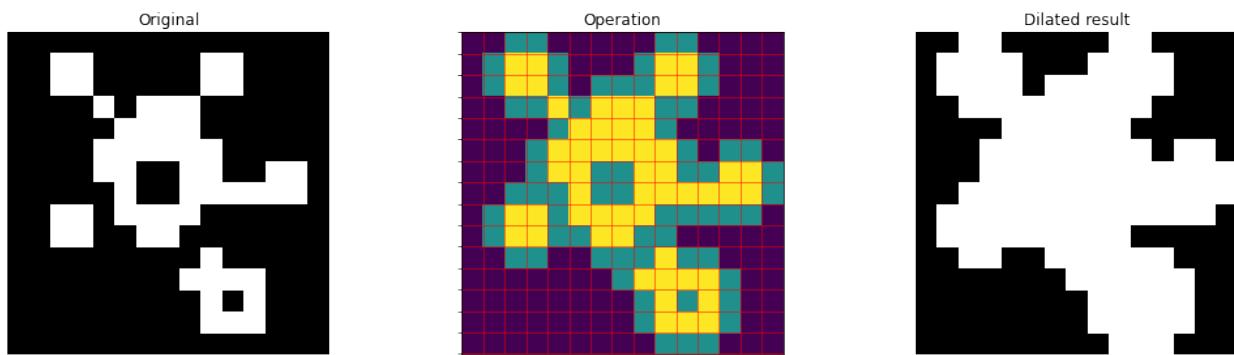
```
dimg=morph.dilation(img,[[0,1,0],[1,1,1],[0,1,0]])

fig, ax = plt.subplots(1,3,figsize=(15,4))

ax[0].imshow(img,cmap='gray'); ax[0].set_title('Original'); ax[0].axis('off');

ax[1].imshow(img+dimg,cmap='viridis');
ax[1].set_xticks(np.arange(-0.5,img.shape[1],1)); ax[1].set_xticklabels([]);ax[1].set_
yticks(np.arange(-0.55,img.shape[0],1)); ax[1].set_yticklabels([])
ax[1].grid(color='red', linestyle='-', linewidth=0.5); ax[1].grid(True);ax[1].set_
title('Operation')

ax[2].imshow(dimg,cmap='gray'); ax[2].set_title('Dilated result');ax[2].axis('off');
plt.tight_layout()
```



Erosion

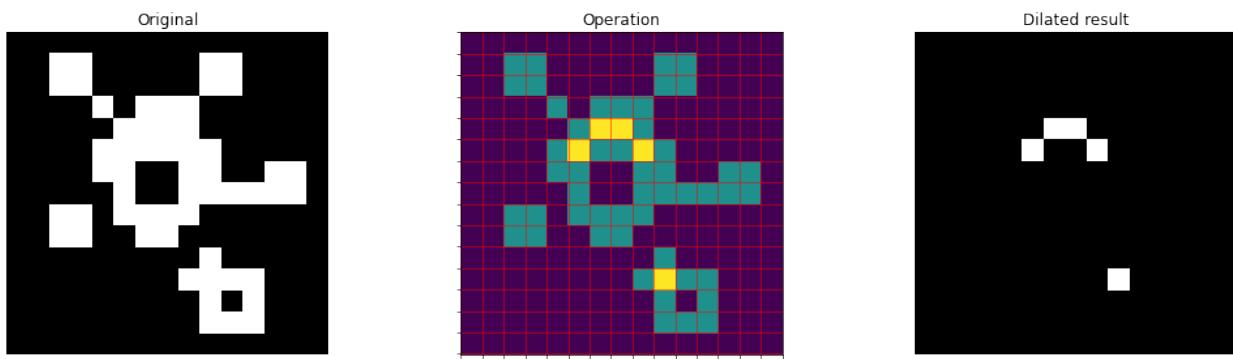
Erosion performs the opposite task reducing the size

```
eimg=morph.erosion(img,[[0,1,0],[1,1,1],[0,1,0]])
fig, ax = plt.subplots(1,3,figsize=(15,4))

ax[0].imshow(img,cmap='gray'); ax[0].set_title('Original'); ax[0].axis('off');

ax[1].imshow(img+eimg,cmap='viridis');
ax[1].set_xticks(np.arange(-0.5,img.shape[1],1)); ax[1].set_xticklabels([]);ax[1].set_
yticks(np.arange(-0.55,img.shape[0],1)); ax[1].set_yticklabels([])
ax[1].grid(color='red', linestyle='-', linewidth=0.5); ax[1].grid(True);ax[1].set_
title('Operation')

ax[2].imshow(eimg,cmap='gray'); ax[2].set_title('Dilated result');ax[2].axis('off');
plt.tight_layout()
```



Opening and Closing

Opening

An erosion followed by a dilation operation

- Peels a layer off and adds a layer on
 - Very small objects and connections are deleted in the erosion and do not return in the dilation thus opened
 - A cube larger than several voxels will have the exact same volume after (conservative)
-

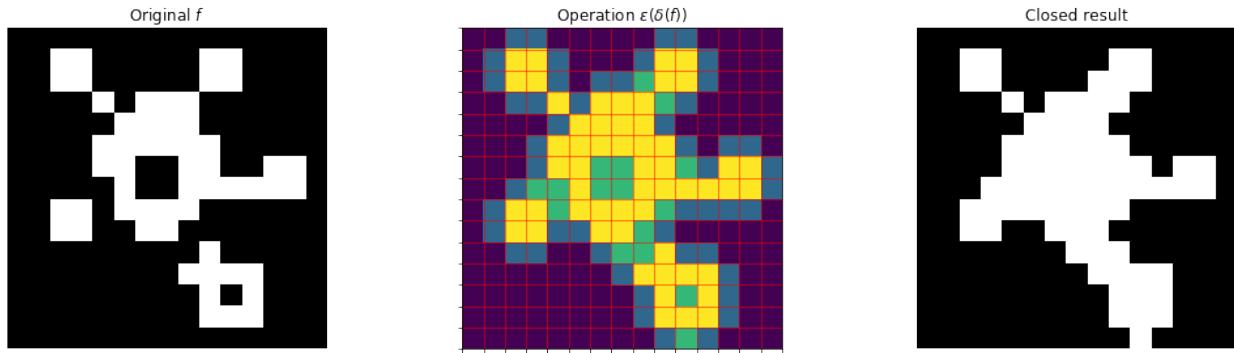
Closing

A dilation followed by an erosion operation

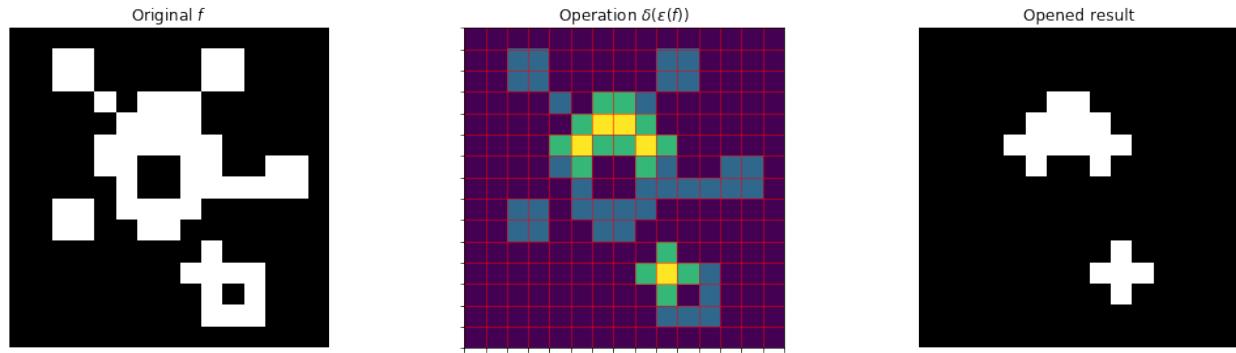
- Adds a layer and then peels a layer off
- Objects that are very close are connected when the layer is added and they stay connected when the layer is removed thus the image is closed
- A cube larger than one voxel will have the exact same volume after (conservative)

Morphological Closing

```
plt.figure(figsize=[15, 4])
plt.subplot(1, 3, 1)
plt.imshow(img, cmap='gray')
plt.axis('off')
plt.title('Original $f$')
plt.subplot(1, 3, 2)
plt.imshow(img+dimg+cimg, cmap='viridis')
plt.title('Operation $\epsilon(\delta(f))$')
plt.xticks(np.arange(-0.5, img.shape[1], 1), labels=[])
plt.yticks(np.arange(-0.55, img.shape[0], 1), labels=[])
plt.grid(color='red', linestyle='-', linewidth=0.5)
plt.grid(True)
plt.subplot(1, 3, 3)
plt.imshow(cimg, cmap='gray')
plt.title('Closed result')
plt.axis('off')
plt.tight_layout()
```



```
plt.figure(figsize=[15, 4])
plt.subplot(1, 3, 1)
plt.imshow(img, cmap='gray')
plt.axis('off')
plt.title('Original $f$')
plt.subplot(1, 3, 2)
plt.imshow(img+eimg+oimg, cmap='viridis')
plt.xticks(np.arange(-0.5, img.shape[1], 1), labels=[])
plt.yticks(np.arange(-0.55, img.shape[0], 1), labels=[])
plt.grid(color='red', linestyle='-', linewidth=0.5)
plt.grid(True)
plt.title('Operation $\delta(\epsilon(f))$')
plt.subplot(1, 3, 3)
plt.imshow(oimg, cmap='gray')
plt.axis('off')
plt.title('Opened result')
plt.tight_layout()
```



Pitfalls with Segmentation

Partial Volume Effect

- The [partial volume effect](#) is the name for the effect of discretization on the image into pixels or voxels.
- Surfaces are complicated, voxels are simple boxes which make poor representations
- Many voxels are only partially filled, but only the voxels on the surface
- Removing the first layer alleviates issue

When is a sphere really a sphere?

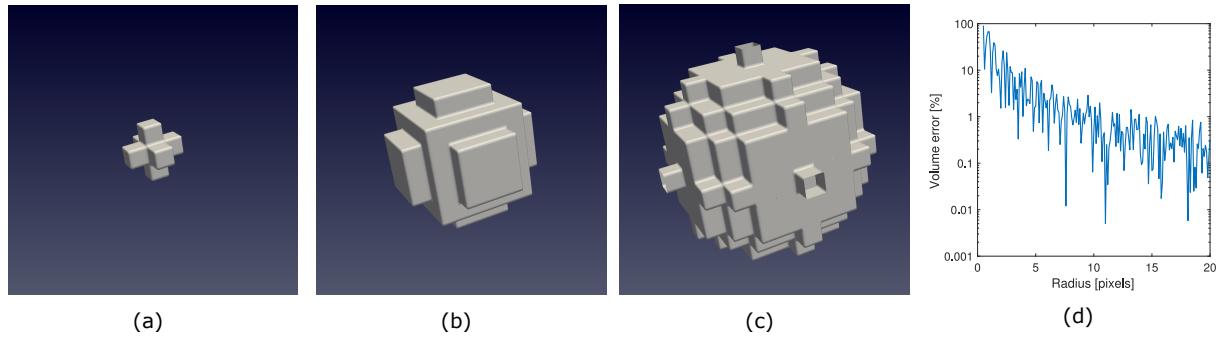


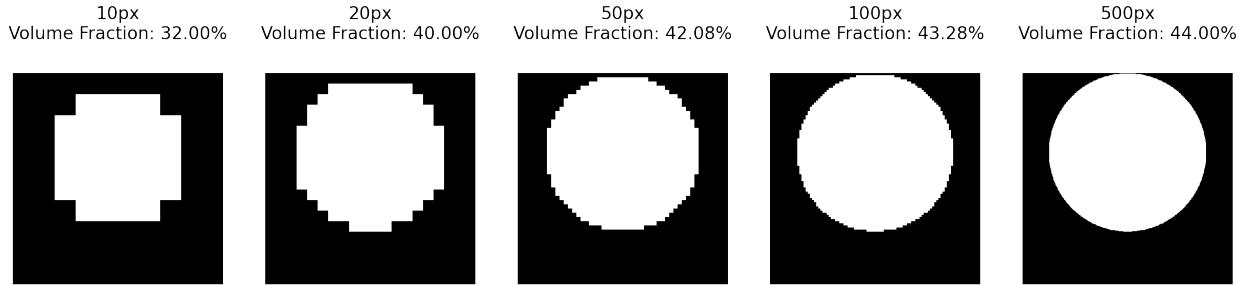
Fig. 12.1: Discrete spheres with increasing radius.

```
from scipy.ndimage import zoom
import numpy as np
import matplotlib.pyplot as plt
from skimage.io import imread
%matplotlib inline
step_list = [10, 20, 50, 100, 500]
fig, m_axs = plt.subplots(1, len(step_list), figsize=(15, 5), dpi=200)
for c_ax, steps in zip(m_axs, step_list):
    x_lin = np.linspace(-1, 1, steps)
    xy_area = np.square(np.diff(x_lin)[0])
    xx, yy = np.meshgrid(x_lin, x_lin)
    test_img = (np.square(xx)+np.square(yy+0.25)) < np.square(0.75)
```

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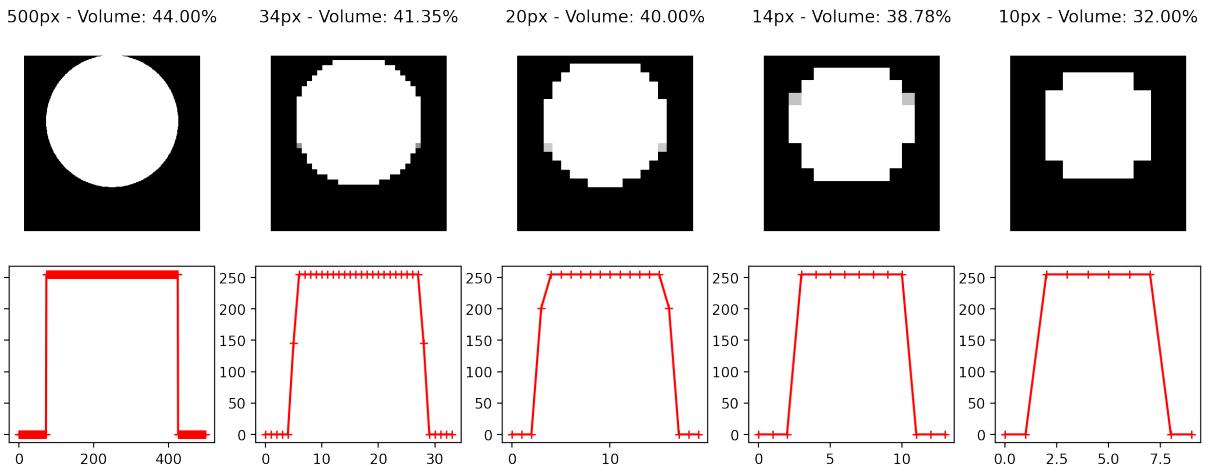
```
c_ax.matshow(test_img, cmap='gray')
c_ax.set_title('%.dpx\nVolume Fraction: %2.2f%%' %
               (steps, 100*np.sum(test_img)/np.prod(test_img.shape)))
c_ax.axis('off')
```



Rescaling

We see the same effect when we rescale images from 500x500 down to 15x15 that the apparent volume fraction changes

```
zoom_level = [1, 0.067, 0.039, 0.029, 0.02]
fig, m_axs = plt.subplots(2, len(zoom_level), figsize=(15, 5), dpi=200)
for (c_ax, ax2), c_zoom in zip(m_axs.T, zoom_level):
    c_img = zoom(255.0*test_img, c_zoom, order=1)
    c_ax.matshow(c_img, cmap='gray')
    c_ax.set_title('%.dpx - Volume: %2.2f%%' %
                   (c_img.shape[0], 100*np.sum(c_img > 0.5)/np.prod(c_img.shape)))
    c_ax.axis('off')
    ax2.plot(c_img[c_img.shape[0]//2], 'r+-')
```



Summary