Laboratory 1 Part 2: Introduction to Imaging

Objectives

- Make your imaging system more quantitative - Learn the focal length of your imaging system - Redesign your microscope for a specific magnification

Introduction

Scientific imaging typically requires quantitative measures. “Images are numbers” and those numbers are often significant to scientific questions. Often image values obtained by a camera are expressed in Arbitrary Digital Units (ADU), which are a function of the input light, detector sensitivity and conversion factors, and analog-to-digital conversion. You have already seen that you have a 10-bit camera whose values attain a maximum of 1023. However, scientifically, these values tend not to have a lot of absolute meaning without additional calibration. Similarly, the size and sampling of your image are dependent on how you have set up your optical system. In the following portions of the lab, you will calibrate ADU to have a scientific meaning (transmissivity) as well as establish the size and sampling of your image (so that you can label the actual sizes of features in your image).

Make your pixel values quantitative

Use the nominal microscope set up from Part 1 of the lab (including aperture wide open). Place a slide in the system and ensure that exposure and illumination settings are good. Use one of the “no filter” settings on the filter wheel (we won’t use filters for the remainder of this lab). Similarly, focus the image and call this image $Y$.

In [1]:

```
# Import and instantiate all classes
from lighting import Lighting
from pololu import Pololu
from camera import Camera
l = Lighting()
m = Pololu()
c = Camera()
l.set_intensity(10)
c.open()
f_wheel,stage_y,stage_x,ap,stage_z = 1,2,3,4,5
m.set_position(ap, 2496, blocking = True) # wide aperture
```

```
Light control initialized successfully.
Servo control initialized successfully.
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ThorCam opened successfully.
```

In [2]:

```
import numpy as np
import matplotlib.pyplot as plt
from IPython.display import display, clear_output
```

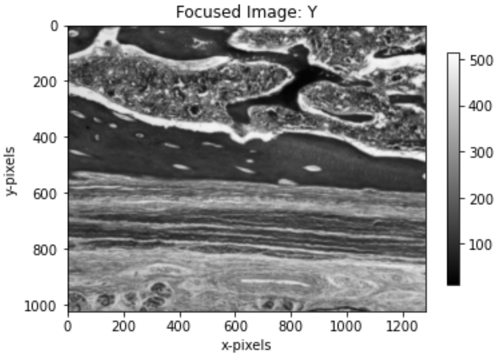
In [37]:

```
m.set_position(f_wheel, 1540, blocking = True) # no filter
m.set_position(stage_y, 500, blocking = True)
m.set_position(stage_x, 1000, blocking = True)
m.set_position(stage_z, 700, blocking = True)
c.set_exposure(10) # good exposure

Y = c.capture().copy()
x = plt.imshow(Y, cmap = "gray")
plt.title("Focused Image: Y")
plt.xlabel("x-pixels")
plt.ylabel("y-pixels")
plt.colorbar(x, shrink=0.8)
```

Out[37]:

```
<matplotlib.colorbar.Colorbar at 0x1b493eff910>
```



We would like to make the pixel values quantitative. For example, pixel values should denote the transmissivity of the sample (which can range from 0-100% transmission of the incident light). To obtain such quantitation, the system must be calibrated – e.g. what measurement values correspond to what transmission. To perform this calibration, we conduct two specialized image captures:
- “Dark” images – Turn off your LED illuminator and close the aperture all the way to prevent light from reaching the camera. Collect another set of 30 images. Average these and call this image D.
- “Air-only” images – Move away from the slide (as shown in the picture below) and collect a series of 30 images with nothing in the system. Find the average of these 30 images (to reduce noise). Call this image A.

Data collection: A, D, and Y
1. Mathematically (using A, D, and Y), what is the expression to obtain quantitative transmission data?
Write the expression and its derivation below.

In order to calculate the image transmission data we first need to calibrate the scale for what it means to have 0% transmission and 100% transmission. We will assume that image D represents 0% and image A represents 100%. In order to obtain the percent transmission, we would divide image Y pixel values by the difference between the pixel values of image A and D so that we are dividing by the total possible range of pixel values for a given pixel. Therefore, we can get percent transmission by getting some image T = (Y - D) / (A - D) for each pixel in T

In order to obtain quantitative transmission data

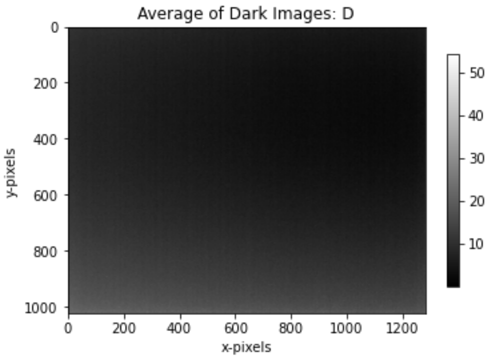
In [20]:

```
l.set_intensity(0)
m.set_position(ap, 992,blocking = True)
dark_imgs = []
for i in range(30):
    dark_imgs.append(c.capture().copy())

D = np.stack(dark_imgs)
D = np.mean(D, axis = 0) # mean of dark images
x = plt.imshow(D, cmap = "gray")
plt.title("Average of Dark Images: D");
plt.xlabel("x-pixels");
plt.ylabel("y-pixels");
plt.colorbar(x, shrink=0.8)
```

Out[20]:

```
<matplotlib.colorbar.Colorbar at 0x1b4915a0ac0>
```



In [23]:

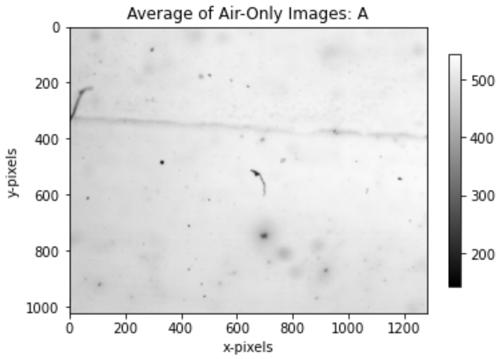
```
l.set_intensity(10)
m.set_position(ap, 2496, blocking = True)
m.set_position(stage_y, 1000, blocking = True)
m.set_position(stage_x, 1000, blocking = True)

air_imgs = []
for i in range(30):
    air_imgs.append(c.capture().copy())

A = np.stack(air_imgs)
A = np.mean(A, axis = 0) # mean of dark images
x = plt.imshow(A, cmap = "gray")
plt.title("Average of Air-Only Images: A");
plt.xlabel("x-pixels");
plt.ylabel("y-pixels");
plt.colorbar(x, shrink=0.8)
```

Out[23]:

```
<matplotlib.colorbar.Colorbar at 0x1b490f84640>
```



2. Create an image of your slide with quantitative transmission values and a colorbar, and show it below:

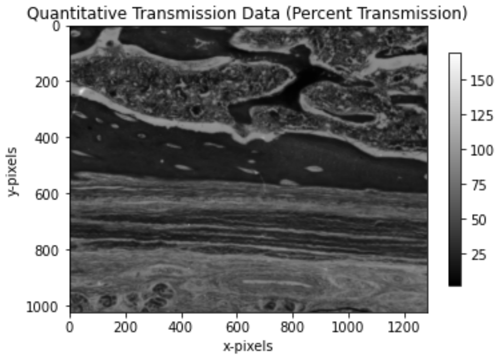
In [42]:

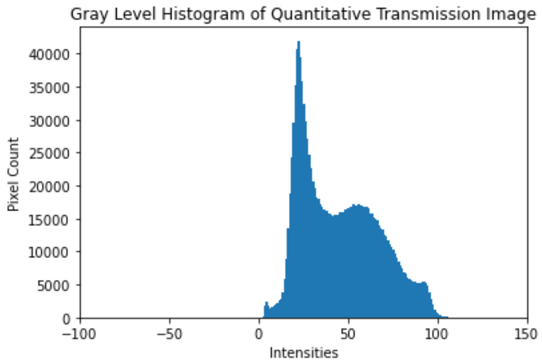
```
qt = ((Y-D)/(A-D)) *100
x = plt.imshow(qt, cmap = "gray")
plt.title("Quantitative Transmission Data (Percent Transmission)");
plt.xlabel("x-pixels");
plt.ylabel("y-pixels");
plt.colorbar(x, shrink=0.8)

plt.figure()
plt.hist(qt.ravel(),1024,[0,1024]);
plt.ylabel("Pixel Count");
plt.xlabel("Intensities");
plt.title("Gray Level Histogram of Quantitative Transmission Image");
plt.xlim(-100,150)
```

Out[42]:

```
(-100.0, 150.0)
```





In [ ]:

Find the magnification of your microscope (Make your axes quantitative)

The actual pixel size of your DCC3240M camera is 5.3 $\mu$m. However, there is magnification between the object you are imaging and the image that falls on the sensor. To find the magnification, you need to measure the size of a known target in pixels.

We will be using the resolution target on your microscope. Move the target so that you can see the 100 $\mu$m grid (pink circle below, 100 $\mu$m is the distance per grid), and make sure it is in focus.

Use Jupyter interactive plotting function (refer to Lab 0), and estimate the size of a pixel in terms of the actual slide feature size in the object plane. You can read the coordinates of the pixel the cursor is hovering over at the bottom of the plot.
Counting pixels over several squares should improve your estimate.

In-class data collection: Image the 100 $\mu$m grid

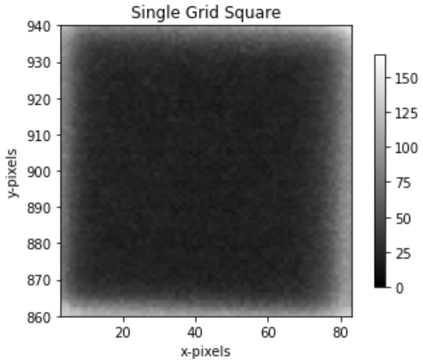
1. Show a zoomed image of the 100 $\mu$m grid and the distance you used to compute the pixel size:

In [27]:

```
# computer: imaging-8
c.set_exposure(10)
m.set_position(stage_y, 1500, blocking = True)
m.set_position(stage_x, 1030, blocking = True)
m.set_position(stage_z, 1700, blocking = True)
x = plt.imshow(c.capture().copy(), cmap = "gray")
plt.title("Single Grid Square");
plt.xlabel("x-pixels");
plt.ylabel("y-pixels");
plt.colorbar(x, shrink=0.8)
plt.xlim(3,83) # x-values for one square
plt.ylim(860,940) # y-values for one square
```

Out[27]:

```
(860.0, 940.0)
```

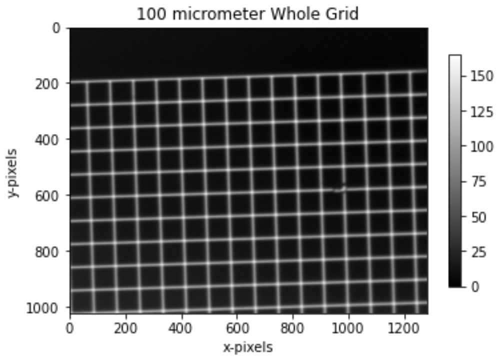


In [29]:

```
x = plt.imshow(c.capture().copy(), cmap = "gray")
plt.title("100 micrometer Whole Grid");
plt.xlabel("x-pixels");
plt.ylabel("y-pixels");
plt.colorbar(x, shrink=0.8)
```

Out[29]:

```
<matplotlib.colorbar.Colorbar at 0x271e73e64f0>
```



The grid shown above is the 100 micrometer grid, the first image is the zoomed-in image of a single box in the grid. The stage positions for this grid on imaging lab 8 are: stage_x = 1030, stage_y = 1500, and stage_z = 1700. The length of each side of the square is 100 micrometers so by using the graph and the zoomed in image we determined that 80 pixels is 100 micrometers.

2. What is the size of one pixel in the object plane?

1 pixel = 100 micrometers / 80 pixels = 1.25 micrometers.

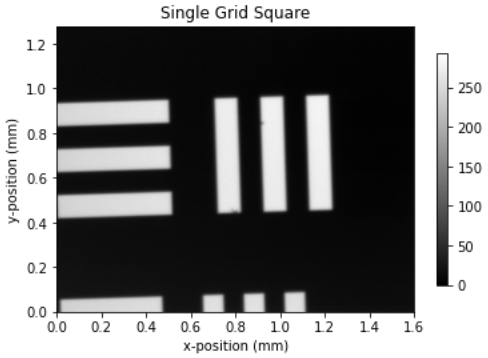
3. Magnification is the ratio of the size of the detected image to the size of the real object. What is the magnification of your system?

1 pixel = 5.3 micrometers (system without magnification) 1 pixel = 1.25 micrometers (system with maginification) magnification: 5.3 / 1.25 = 4.24

4. Take an image of the line pairs region (green circle above) in the resolution target and paste it below with a color bar and proper axis labels (mm).

In [50]:
```
# computer: imaging-8
c.set_exposure(10)
m.set_position(stage_y, 1640, blocking = True)
m.set_position(stage_x, 810, blocking = True)
m.set_position(stage_z, 1700, blocking = True)
img = c.capture().copy()
x = plt.imshow(img, cmap = "gray", extent = [0,1.25/1000 * img.shape[1], 0, 1.25/1000*img.shape[0]])
plt.title("Single Grid Square");
plt.xlabel("x-position (mm)");
plt.ylabel("y-position (mm)");
plt.colorbar(x, shrink=0.8)
```

Out[50]:
```
<matplotlib.colorbar.Colorbar at 0x271eb5b6160>
```



5. What are the finest line pairs that you can resolve? Show a zoomed image including the line pairs above and below this limit. (The following table will help you find the lp/mm for each target. Values are in lp/mm. The element number is labeled on the left of each line pair target; the group number is on top.)

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Devise a strategy to measure the focal length of your lens

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In [ ]:
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In [12]:
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# computer: imaging-8
c.set_exposure(10)
m.set_position(stage_x, 1700, blocking= True)
m.set_position(stage_y, 2000, blocking = True)
m.set_position(stage_z, 1000, blocking = True)
x = plt.imshow(c.capture().copy(), cmap = "gray")
plt.title("Single Grid Square");
plt.xlabel("x-pixels");
plt.ylabel("y-pixels");
plt.colorbar(x, shrink=0.8)
```

Out[12]:
```
<matplotlib.colorbar.Colorbar at 0x1bb79218cd0>
```

In [27]:
```
# computer: imaging-8
x = plt.imshow(c.capture().copy(), cmap = "gray")
plt.title("Single Grid Square");
plt.xlabel("x-pixels");
plt.ylabel("y-pixels");
plt.colorbar(x, shrink=0.8)
plt.xlim(253, 308) ## 55 pixels x direction
plt.ylim(852, 907) ## 55 pixels y direction
```

Out[27]:
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(852.0, 907.0)
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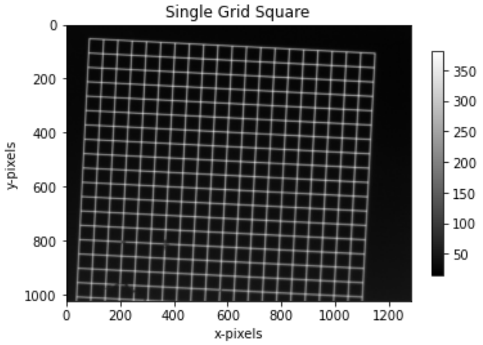

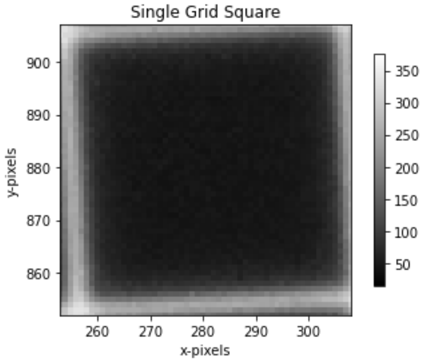

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