HW_6_AD

November 7, 2024

```
[185]: import numpy as np
       import matplotlib.pyplot as plt
       from scipy.special import j1
       from tqdm.auto import tqdm
       from skimage import io
       from matplotlib.animation import FuncAnimation
       from skimage.filters import gaussian
[159]: def showim(im_array, figsize=(4, 4), show_hist=False, nbins=None,
        ⇒bin_width=None, cmap='gray', vmin=None, vmax=None, titles=None):
           if isinstance(im_array, (list, tuple)):
               n_images = len(im_array)
               fig_width, fig_height = figsize
               plt.figure(figsize=(fig_width * n_images, fig_height))
               for idx, img in enumerate(im_array):
                   plt.subplot(1, n_images, idx + 1)
                   plt.imshow(img, cmap=cmap, vmin=vmin, vmax=vmax)
                   if titles and isinstance(titles, (list, tuple)) and len(titles) ==__
        \rightarrown_images:
                       plt.title(titles[idx])
                   elif titles and isinstance(titles, str):
                       plt.title(titles)
                   plt.axis('off')
               plt.tight_layout()
               plt.show()
           else:
               plt.figure(figsize=figsize)
               if show_hist:
                   plt.subplot(1, 2, 1)
                   plt.imshow(im_array, cmap=cmap, vmin=vmin, vmax=vmax)
```

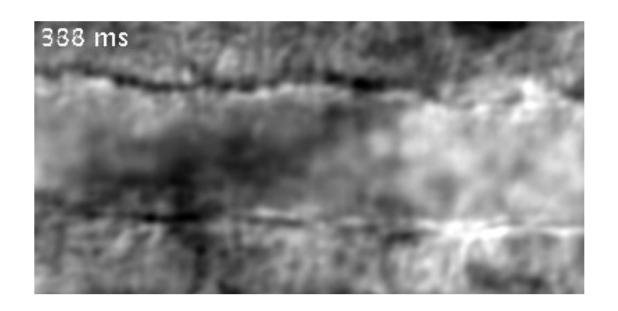
```
if titles and isinstance(titles, str):
                plt.title(titles)
            plt.axis('off')
            plt.subplot(1, 2, 2)
            im_flattened = im_array.ravel()
            min_val = np.floor(im_flattened.min())
            max_val = np.ceil(im_flattened.max())
            if bin_width is not None:
                bins = np.arange(min_val, max_val + bin_width, bin_width)
            elif nbins is not None:
                bins = nbins
            else:
                bins = int(max_val - min_val)
            plt.hist(im_flattened, bins=bins, color='black')
            plt.xlabel('Intensity Value')
            plt.ylabel('Frequency')
            plt.title('Image Intensity Histogram')
        else:
            plt.imshow(im_array, cmap=cmap, vmin=vmin, vmax=vmax)
            if titles and isinstance(titles, str):
                plt.title(titles)
            plt.axis('off')
        plt.tight_layout()
        plt.show()
def animate_stack(stack, fname = "animated_stack", fps = 10):
    fig, ax = plt.subplots()
    im = ax.imshow(stack[0], cmap='gray', vmin=0, vmax=255)
    ax.axis('off')
    def update(frame):
        im.set_data(stack[frame])
        ax.set_title(f"Frame {frame}")
        return [im]
    ani = FuncAnimation(fig, update, frames=len(stack), interval=100, blit=True)
    plt.show()
    ani.save(f"{fname}.gif", writer="pillow", fps=fps)
def surfplot(imarr, figsize = (11,7), cmap = 'viridis', show = True):
```

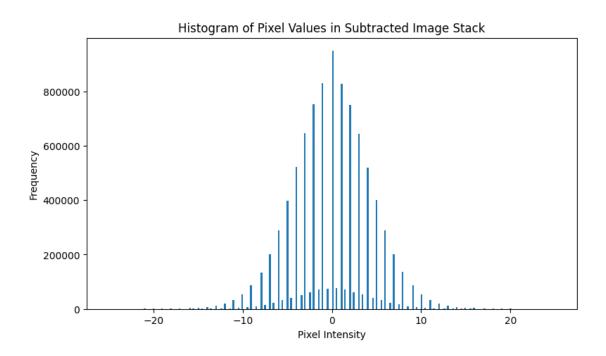
```
x = np.arange(imarr.shape[1])
y = np.arange(imarr.shape[0])
X , Y = np.meshgrid(x, y)

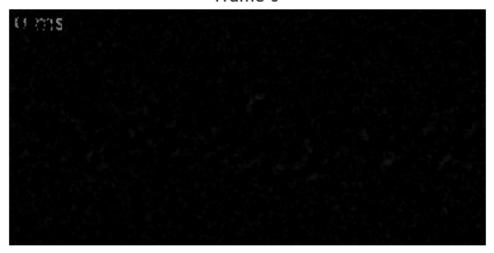
if show == True:

    fig = plt.figure(figsize=figsize)
    ax = fig.add_subplot(111, projection='3d')
    ax.plot_surface(X, Y, imarr, cmap=cmap)
    ax.set_xlabel('X')
    ax.set_ylabel('Y')
    ax.set_zlabel('Pixel Intensity')
    plt.show();
```

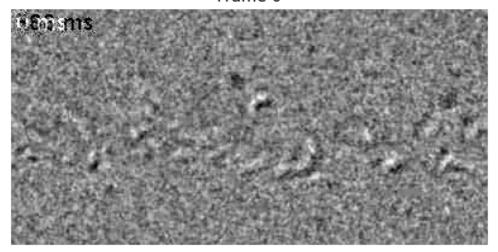
A) Subtract the median from each pixel in each frame. (Note: you needn't loop through each pixel! Median functions can act on a specified array dimension, and you can subtract one 2D array from another, all at once.) Submit (i) the median image, and (ii) a histogram of all the pixel values in the subtracted 3D image array.







Frame 0



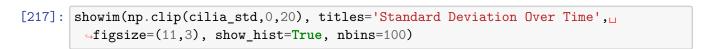
C)

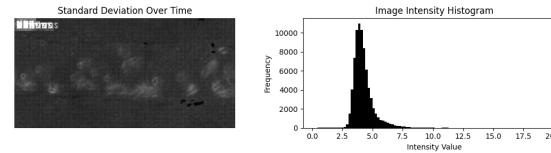
Calculate the standard deviation of each (x, y) pixel over time, creating a single 2D image that

shows this. In other words, pixel intensity [i,j] of the 2D image should be the standard deviation of the pixels intensities at position [i,j] in the 3D image series. Submit this image. (Hint: Making the array should be one line of code.)

Standard Deviation Over Time

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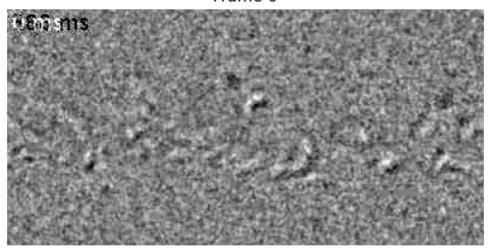




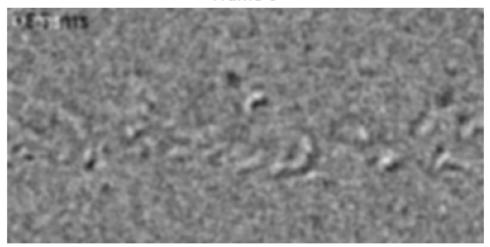
D) Hopefully from watching the result of (a) you can see filament-like things are moving. Can you enhance this further? How could you weight different pixels differently, or do some sort of averaging, or otherwise make things clearer? Think about this, play around with the arrays, and submit the best thing you can come up with as multipage TIFF image, along with a short description. Obviously, there is no "correct answer.

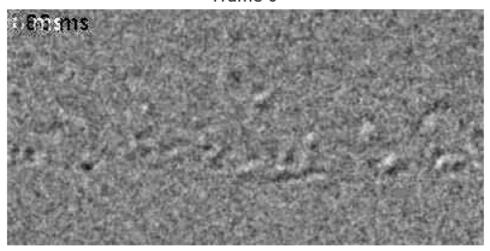
```
[296]: def subtract_moving_average(stack, window_size=3):
           if window_size % 2 == 0:
               raise ValueError("Window size should be odd.")
           pad_width = window_size // 2
           padded_stack = np.pad(stack, ((pad_width, pad_width), (0, 0), (0, 0)),__
        →mode='reflect')
           result_stack = np.empty_like(stack)
           for t in range(stack.shape[0]):
               moving_average = np.mean(padded_stack[t:t + window_size], axis=0)
               result_stack[t] = moving_average
           return result_stack
 []: def subtract_moving_average_conditional(stack, window_size=3,__
        ⇒std_dev_threshold=10):
           std_dev_map = np.std(stack, axis=0)
           low_variability_mask = std_dev_map < std_dev_threshold</pre>
           pad_width = window_size // 2
           padded_stack = np.pad(stack, ((pad_width, pad_width), (0, 0), (0, 0)),__
        →mode='reflect')
           result_stack = np.empty_like(stack)
           for t in range(stack.shape[0]):
               moving_average = np.mean(padded_stack[t:t + window_size], axis=0).
        →astype(np.uint8)
               result_stack[t] = stack[t]
               result_stack[t][low_variability_mask] = __
        →moving_average[low_variability_mask]
           return result_stack
 []: window_size = 11
       moving_average_conditional= subtract_moving_average_conditional(rescaled_stack,_
       ⇒window_size=window_size, std_dev_threshold=3)
       animate_stack(moving_average_conditional, 'moving_avg_conditional')
       low_pass_moving_avg = np.array([(gaussian(frame, sigma=1.5)*255).astype(np.
        ouint8) for frame in moving_average_conditional])
       animate_stack(low_pass_moving_avg, 'moving_avg_conditional_lowpass')
```

Frame 0

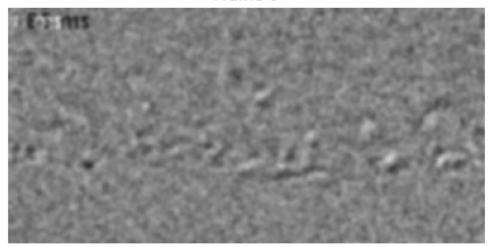


Frame 0

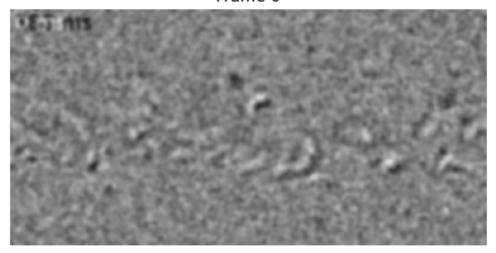




Frame 0



(Official Attempt)



The best method I could come up with, without compeltely obfuscating the movement/shape of the cilia, was a simple gaussian filter. I did attempt setting the pixels as their moving average depending on their std deviation, since pixels with higher standard deviations more likely correlate to movement and thus we could average out the noisy non-cilia pixels. However, the main issue isn't the surrounding pixels but the pixels within the path of the moving cilia, (ones with high std dev). Maybe some logic could be done such as edge detection or checking how quickly the pixel value has changed with respect to time (i.e. within the past nstacks) and make that binarize the image by such a intensity rate of change threshold. I attempted something like it but I think that just takes a lot of fine-tuning for it to be successful, see my attempts below.

```
[281]: def binary_mask_per_frame(stack, threshold):
    std_dev_map = np.std(stack, axis=0)
    mask = (std_dev_map > threshold).astype(np.uint8)
    binary_mask_stack = stack * mask

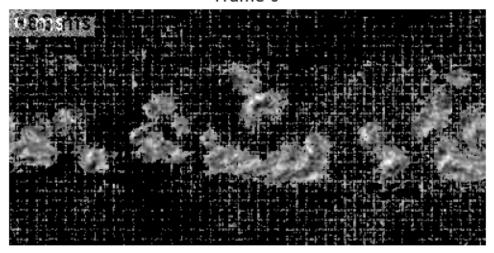
    return binary_mask_stack

threshold = 28

binary_mask = binary_mask_per_frame(rescaled_stack, threshold)
print(binary_mask.shape)

animate_stack(binary_mask)
```

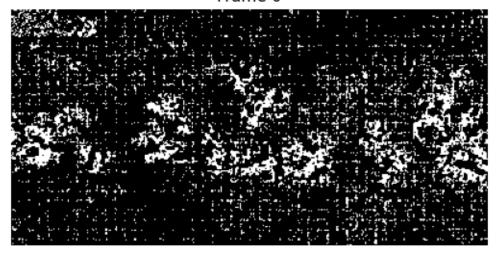
(126, 196, 396)



```
[295]: def compute_rate_of_change(stack, nstacks=3, threshold=None, upper_bound=255):
           diff_stack = np.abs(np.diff(stack, axis=0).astype(np.float64))
           rate_of_change = np.zeros_like(stack[:-nstacks + 1], dtype=np.float64)
           for i in range(nstacks - 1):
               rate_of_change += diff_stack[i:stack.shape[0] - nstacks + 1 + i]
           rate_of_change /= (nstacks - 1.0)
           if threshold is not None:
               output1 = (rate_of_change > threshold).astype(np.uint8)
               output2 = (rate_of_change < upper_bound).astype(np.uint8)</pre>
               output = (output1 & output2)
           else:
               output = rate_of_change.astype(np.uint8)
           return output
       bin_stack = compute_rate_of_change(binary_mask, nstacks=2, threshold=0,__

upper_bound=100)*255

       animate_stack(bin_stack)
```



D) Do the cilia look like they are moving "back and forth" with the same form, or not? (In other words, does the shape of a cilium look the same on the forward and reverse stroke? Does the movie look the same if played forward and backward?) I found a region near the center to be clearest to see this. Answer as best you can – showing some snapshots might help, but you don't have to.

The cilia do look like they are moving back and forth with the same form, with the shape being the same on the forward and backwawrd stroke.

Problem 2

```
[309]: import numpy as np
       import matplotlib.pyplot as plt
       from scipy.optimize import minimize
       N = 20
       x = np.linspace(-3, 4, N)
       x_{true}, b_{true} = 0.75, 2.2
       A = 3 * np.abs(x - x_true) ** b_true + 4
       y = np.random.poisson(A)
       def objective(params, x, y):
           x0, b = params
           A_{model} = 3 * np.abs(x - x0) ** b + 4
           return np.sum(A_model - y * np.log(A_model))
       initial_guess = [1.0, 2]
       result = minimize(objective, initial_guess, args=(x, y), bounds=((0, None), (0, __

None)))
       x0_est, b_est = result.x
```

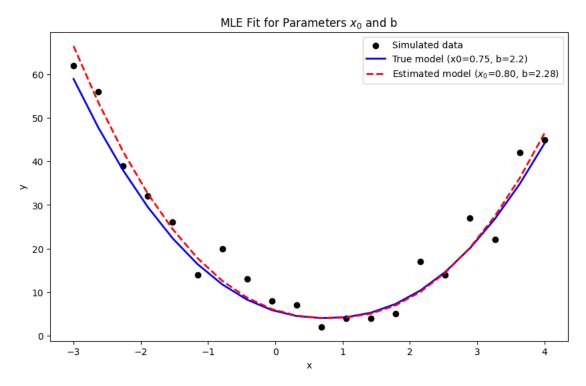
```
print(f'Estimated x0: {x0_est}, Estimated b: {b_est}')
```

Estimated x0: 0.7970237011799536, Estimated b: 2.2759347641051666

B) How close are the estimated x_0 and b to the true values? Run your code a few times, and "by eye" comment on the accuracy. (Or if you want, run it a lot and calculate the RMSE.)

The values are very similar to the original (true) values used to generate the data, indicating good performance of the MLE.

Plot the simulated y vs. x datapoints together with the function $3|x - x_0|b + 4$ for both the true and estimated parameter values. Submit the plot.

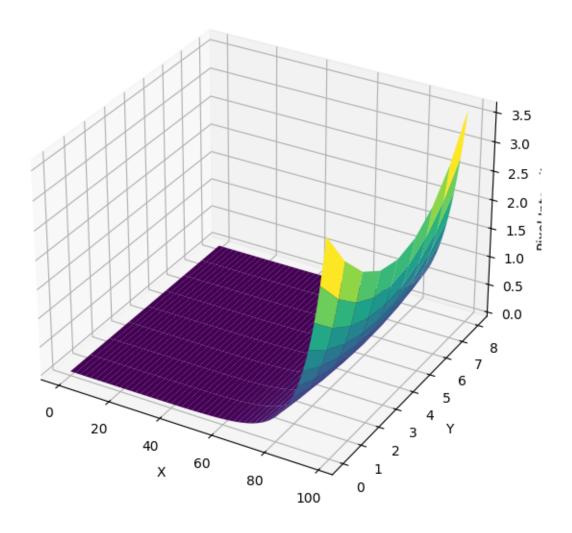


D) Calculate the objective function for a grid of parameter values x0 = 0.1 to 10 in steps of 0.1, and b = 0.1 to 10 in steps of 0.1. Submit a plot of this – it could be a surface plot, or a 2D plot with color indicating the value of the objective function.

```
[314]: x0_vals = np.arange(0.1, 1.0, 0.1)
b_vals = np.arange(0.1, 10, 0.1)
objective_grid = np.zeros((len(x0_vals), len(b_vals)))

for i, x0 in enumerate(x0_vals):
    for j, b in enumerate(b_vals):
        objective_grid[i, j] = objective([x0, b], x, y)

surfplot(objective_grid);
```



3 Centroid localization timing. (2 pts.)

Re-run your center-of-mass (centroid) localization code on simulated 2D single-molecule images as in Homework 5. Use the parameters described above, and have the true positions be random numbers between -0.05 m and 0.05 m in x and y. Assess how long per image the centroid localization takes – in other words, simulate M images and note total time / M. (Note the timing functions we used in HW3) Note that you should not include the time needed to create the simulated images. First create a (3D) array of simulated images and then loop through these, running your centroid localization function on each "slice." Submit one number: the centroid computation time per image.

- 7×7 px images with scale = 100 nm/px (i.e. 0.1 m/px), = 510 nm, NA = 0.9
- Number of photons = 1000
- M = 100 simulated images for each condition

```
[315]: def calculate centroid(intensities, camera_scale = None, origin center=False):
           HHHH
           Calculate the centroid (center of mass) for 1D or 2D intensity arrays,
           with an option to shift the origin to the center of the array.
           Parameters:
           intensities (np.array): Array of intensity values, either 1D or 2D.
           origin_center (bool): If True, shifts the origin to the center of the array.
                                 If False, uses the bottom-left corner as the origin.
           Returns:
           tuple: The centroid position. For 1D, returns (x centroid,).
                  For 2D, returns (x_centroid, y_centroid).
           if intensities.ndim == 1:
               # 1D case
               n = len(intensities)
               positions = np.arange(n)
               if origin_center:
                   center = (n - 1) / 2
                   positions = positions - center
               x_centroid = np.sum(positions * intensities) / np.sum(intensities)
               if camera_scale:
                   return x_centroid*camera_scale
               else:
                   return x_centroid
           elif intensities.ndim == 2:
               # 2D case
               m, n = intensities.shape
               x_positions = np.arange(n)
               y_positions = np.arange(m)
```

```
if origin_center:
            x_center = (n - 1) / 2
            y_center = (m - 1) / 2
            x_positions = x_positions - x_center
            y_positions = y_positions - y_center
        x_weights = np.sum(intensities, axis=0)
        y_weights = np.sum(intensities, axis=1)
        x_centroid = np.sum(x_positions * x_weights) / np.sum(intensities)
        y_centroid = np.sum(y_positions * y_weights) / np.sum(intensities)
        if camera_scale:
            return (x_centroid*camera_scale, y_centroid*camera_scale)
            return (x_centroid, y_centroid)
    else:
        raise ValueError("Only 1D and 2D arrays are supported")
def sim_ps(N_camera, wavelength, NA, camera_scale, fine_scale, N_photon, center_
 \hookrightarrow= None, B = None):
    block_size = int(camera_scale / fine_scale)
    fine_grid_N = N_camera * block_size
    x = np.linspace(-fine_grid_N//2, fine_grid_N//2, fine_grid_N) * fine_scale
    y = np.linspace(-fine_grid_N//2, fine_grid_N//2, fine_grid_N) * fine_scale
    X, Y = np.meshgrid(x, y)
    if center:
        xc, yc = center
    else: xc, yc = 0, 0
   r = np.sqrt((X - xc)**2 + (Y - yc)**2)
    v = (2 * np.pi / wavelength) * NA * r
    psf_fine = np.zeros_like(v)
    psf fine[v == 0] = 1
    psf_fine[v != 0] = 4 * (j1(v[v != 0]) / v[v != 0])**2
    psf_fine = psf_fine / psf_fine.sum()
    psf_camera = np.zeros((N_camera, N_camera))
    for i in range(N_camera):
        for j in range(N_camera):
```

```
[316]: import time
       N_{camera} = 7
       wavelength = 0.51
       NA = 0.9
       camera_scale = 0.1
       fine_scale = 0.01
       N_{photons} = 1000
       bg = 2
       M = 100
       ps_stack = []
       for _ in range(M):
           center_x = np.random.uniform(-0.05,0.05)
           center_y = np.random.uniform(-0.05,0.05)
           center = (center_x,center_y)
           new_ps = sim_ps(N_camera=N_camera, wavelength=wavelength, NA=NA,__
        ⇒camera_scale=camera_scale,
                     fine_scale=fine_scale, N_photon=N_photons, center = center, B=bg)
           ps_stack.append(new_ps)
       ps_stack = np.array(ps_stack)
       print(ps_stack.shape)
       start_time = time.time()
       for i in range(M):
```

(100, 7, 7)

Average time per centroid calculation: 0.000040 seconds

4 Gaussian MLE for particle localization! (14 pts.)

Write a 2D MLE particle localization function! In other words, write a function that returns the MLE estimate for the particle center (xc, yc) and other parameters given a 2D image in which each pixel has Poisson-distributed random values with a mean that depends on position as:

$$A(x,y)=A_0exp[-\frac{(x-xc)^2+(y-yc)^2}{2\sigma^2}]+B$$

(In other words, a Gaussian approximation to the PSF.) Then apply Gaussian MLE localization to the same sorts of simulated images that you used earlier.

```
[155]: def calculate_MLE(intensities, initial_guess, camera_scale=0.01,
        ⇔origin_center=True):
           N = intensities.shape[0]
           x = np.arange(N)
           y = np.arange(N)
           if origin_center==True:
               x = x - (N - 1) / 2
               y = y - (N - 1) / 2
           X, Y = np.meshgrid(x, y)
           def objective(params, X, Y, data):
               xc, yc, AO, sigma, B = params
               model = A0 * np.exp(-((X - xc)**2 + (Y - yc)**2) / (2 * sigma**2)) + B
               return np.sum(model - data * np.log(np.where(model > 0, model, 1)))
           results = minimize(objective, initial_guess, args=(X, Y, intensities))
           xc_est, yc_est, A0_est, sigma_est, B_est = results.x
           if camera_scale:
               xc_est *= camera_scale
               yc_est *= camera_scale
           return xc_est, yc_est, A0_est, sigma_est, B_est
```

B) Repeat #3, now using MLE localization. Time how long per image it takes.

```
[150]: #xc_est, yc_est, AO_est, sigma_est, B_est
initial_guess = [0,0,100,1,0]
start_time = time.time()

for i in range(M):
    calculate_MLE(ps_stack[i], initial_guess=initial_guess,u)
    camera_scale=camera_scale, origin_center=True)
end_time = time.time()

print(f"Average time per centroid calculation: {(end_time-start_time)/M:.6f}u
    seconds")
```

Average time per centroid calculation: 0.007541 seconds

The MLE certainly takes longer than the centroid calculation, which makes sense. We are doing a minimization problem which involves many more operations (and overhead using imported libraries) in addition to now "finding" 5 parameters instead of the 2 in the centroid calculation

C) Repeat HW5 #3c, now using MLE localization, making histograms for:

Nphotons = 1000, (x0, y0) = (0.0, 0.0) px, (x0, y0) = (0.3, 0.0) px, and (x0, y0) = (-0.3, 0.0) px.

Does the MLE estimate look unbiased?

```
[158]: N_camera = 7
    wavelength = 0.510
    NA = 0.9
    N_photons = 1000
    camera_scale = 0.1
    fine_scale = 0.001
    bg = 10

m = 10000
    center_list = [(0.0, 0.0), (0.03, 0.0), (-0.03, 0.0)]

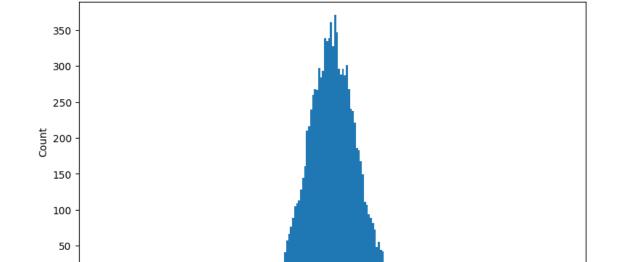
for center in center_list:
        x_error_list = []
        current_center = center

        for i in range(m):
```

```
image = image = sim_ps(N_camera=N_camera, wavelength=wavelength, NA=NA,_
⇔camera_scale=camera_scale,
             fine_scale=fine_scale, N_photon=N_photons,_
MLE = calculate_MLE(image, initial_guess=initial_guess,__

¬camera_scale=camera_scale, origin_center=True)

     x MLE = MLE[0]
     x_error = x_MLE - center[0]
     x_error_list.append(x_error)
  plt.figure(figsize=(9,5))
  plt.hist(x_error_list, bins=100)
  plt.xlim(-0.05, 0.05)
  plt.xlabel('X-Error (um)')
  plt.ylabel('Count')
  plt.title(f"X-Error Distribution with Center: ({center[0]}um,_
```



0.00

X-Error (um)

0.02

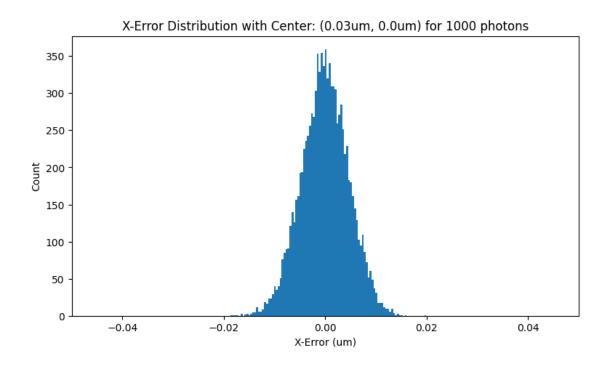
0.04

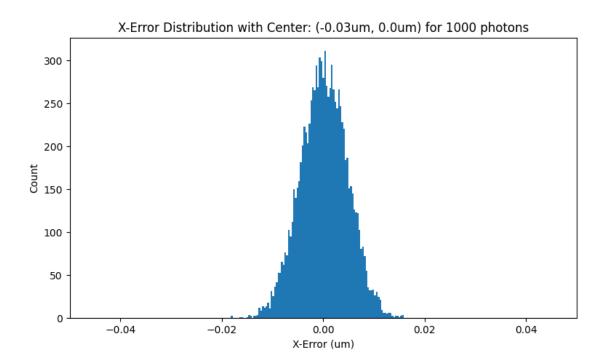
0

-0.04

-0.02

X-Error Distribution with Center: (0.0um, 0.0um) for 1000 photons





The MLE does indeed look unbaised! The distribution of error is comparable in all three of these cases, indicating an unbiased method for finding the center of the point source.

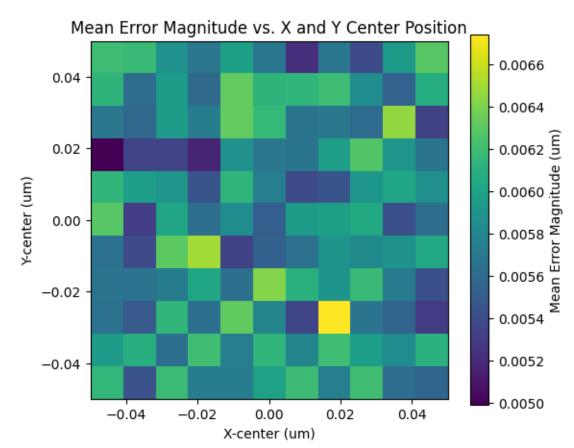
```
[157]: N_{camera} = 7
      wavelength = 0.510
      NA = 0.9
      N_{photons} = 1000
      camera_scale = 0.1
      fine_scale = 0.001
      bg = 10
      m = 100
      center x list = np.linspace(-0.05, 0.05, 11)
      center_y_list = np.linspace(-0.05, 0.05, 11)
      mean_error_magnitude_array = np.zeros((len(center_x_list), len(center_y_list)))
      for i, center_x in tqdm(enumerate(center_x_list), total = len(center_x_list),_u

desc = f"Outer Loop, X Center = {center_x:.4f} ", leave=False):

          for j, center_y in tqdm(enumerate(center_y_list), total=len(center_y_list),
                                   desc=f"Processing Y positions for {center_x:.4f}",
        →leave=False):
              current_center = (center_x, center_y)
              error_magnitude_list = []
              for _ in range(m):
                  image = sim_ps(N_camera=N_camera, wavelength=wavelength, NA=NA,_
        ⇔camera scale=camera scale,
                                 fine_scale=fine_scale, N_photon=N_photons,_
        MLE = calculate_MLE(image, initial_guess=initial_guess,__
        →origin_center=True, camera_scale=camera_scale)
                  x_MLE, y_MLE = MLE[0], MLE[1]
                  error_magnitude = np.sqrt((x_MLE - center_x) ** 2 + (y_MLE -
        ⇔center_y) ** 2)
                  error_magnitude_list.append(error_magnitude)
              mean_error_magnitude_array[i, j] = np.mean(error_magnitude_list)
      plt.figure(figsize=(6, 5))
      c = plt.imshow(mean_error_magnitude_array, extent=[center_x_list[0],_
       ocenter_x_list[-1], center_y_list[0], center_y_list[-1]],
                      cmap='viridis')
      plt.colorbar(c, label="Mean Error Magnitude (um)")
```

```
plt.title("Mean Error Magnitude vs. X and Y Center Position")
plt.xlabel("X-center (um)")
plt.ylabel("Y-center (um)")
plt.show()
```

Outer Loop, X Center = -0.0400 :	0%	0/11 [00:00 , ?it/s]</th
Processing Y positions for -0.050	00: 0%	0/11 [00:00 , ?it/s]</td
Processing Y positions for -0.04	00: 0%	0/11 [00:00 , ?it/s]</td
Processing Y positions for -0.030	00: 0%	0/11 [00:00 , ?it/s]</td
Processing Y positions for -0.020	00: 0%	0/11 [00:00 , ?it/s]</td
Processing Y positions for -0.010	00: 0%	0/11 [00:00 , ?it/s]</td
Processing Y positions for 0.000	0%	0/11 [00:00 , ?it/s]</td
Processing Y positions for 0.010	0%	0/11 [00:00 , ?it/s]</td
Processing Y positions for 0.020	0: 0%	0/11 [00:00 , ?it/s]</td
Processing Y positions for 0.030	0: 0%	0/11 [00:00 , ?it/s]</td
Processing Y positions for 0.0400	0%	0/11 [00:00 , ?it/s]</td
Processing Y positions for 0.0500	0: 0%	0/11 [00:00 , ?it/s]</td



It seems like the error no longer depends on the position, which is great! We want methods that have similar (or unbiased) errors and error distributions independent of the true value, which seems to be what this MLE does.